PHYSIOLOGICAL ZOÖLOGY

A Quarterly Journal of Zoölogical Research

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Frank Rattray Lillie

1870-1947

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PHYSIOLOGICAL ZOÖLOGY

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DOMINANCE ORDER IN *POLISTES* WASPS

L. PARDI

Istituto di Zoologia, Università di Pisa, Italy

From the researches of Schjelderup-Ebbe (1922) until now, studies about the social hierarchy in the vertebrate animals have made considerable progress. It has been possible to enlarge the ideas about hierarchy based upon dominance to all the classes of vertebrates (except the amphibians); also it has been possible, for the majority of the observed species, to individuate some internal and external factors of the phenomenon. Moreover, Allee and his collaborators (e.g., 1939) have had success in modifying the social status of the individuals by experimental treatment with hormones.

As far as the insects and the invertebrates generally are concerned, knowledge about the question has been vague until now; I think I am right when I assert that the existence of a proper social hierarchy in the insects has never been reported until now. For that reason Allee (1945*b*) rightly wrote in a recent review of the subject: "Group organization as we vertebrates show it, is wholly lacking among the lower invertebrates; and, among the social insects, for the most part, it is developed along different lines from the customary pattern in vertebrate groups."

In 1942, I was studying by means of Steiner's method (1932) the work division between fertilized females of *Polistes gallicus* (L.) appropriately marked for recognition of different individuals. These females associate in the spring in founding the same nest (*polygynic association*), and I perceived that the dominance idea, as Schjelderup-Ebbe defined it, could reasonably be extended also to this wasp. Subsequent observations have confirmed my point of view, and I was able to demonstrate the rules on which the hierarchy organization is based and certain of the factors and the consequences of this organization, both among the females associated in the spring and among the workers.

I think it is right to give an ample résumé of these researches (for the most part already published in Italian) (Pardi, 1942, 1946).

THE BIOLOGICAL CYCLE OF *P. gallicus*

At our latitudes the nests of *P. gallicus* (L.) are built in the spring, generally by the beginning of April. The foundation can be monogynic but more frequently, in Italy at least, several females, after their wintering, co-operate in the same nest (*polygynic foundation*). It is known

that the nests are gymnodome and are suspended in cavities of different sorts, mainly under the tiles of roofs. The associated founding females coexist in the nest until the eclosion of the workers, and soon a work distribution is established among them (Heldmann [1936]; Pardi, 1942)[1]—one, the leader female, remains on the nest, lays eggs intensively, does less building than her associates, and, as will appear later, dominates the associates (Pardi, 1942). The other females (auxiliary females) go out more frequently, build, bring solid and liquid food, lay eggs less, and are dominated. Soon the auxiliary females finish laying, and their ovaries gradually become reduced. After the eclosion of the first workers, the auxiliary females are eliminated sooner or later from the association, either because the leader paralyzes them by a sting or because the continuous hostility of the leader reduces them to exhaustion (Pardi, 1942). The leader (which now can be considered the veritable queen of the nest) remains alone with the workers, goes on with laying eggs, and from this moment leaves the nest rarely.

As is known, all the other social functions, except laying eggs, are undertaken by the workers, which build, feed the larvae, regulate the temperature by fanning their wings,[2] defend the nest, and so on. From the eggs of the queen there continue to be born other females with the character of workers and also some males, but the males make their appearance in great number only in August. On the other hand, during this period of time, as the colony is growing and trophic conditions are improving, the females that are born are always larger and have more fat reserves; these females do not maintain the typical behavior of the workers and stay much on the nest.[3] These females, unlike their elder sisters, tolerate copulation and, after fecundation, they live over the winter and found the nests in the next spring.

[1] According to Rau (1938, p. 232), in *P. annularis*, also, one of the founding females is a queen; the others behave as workers.

[2] According to Steiner the queen of *P. dubia* also co-operates in this particular social work by bringing water.

[3] Between the mentioned individuals and the true workers there are all the types of intermediate characteristics.

BEHAVIOR PATTERNS

Lack of equivalence.—In the great majority of the contacts between individuals of the same nest (between two founding females or two workers, or between a representative of each group) the individuals show a different (not equivalent) behavior: one of them behaves as dominant (generally active), the other as dominated (generally passive). For instance, in the most typical and frequent contact, the dominant (Δ) strikes the head of the dominated repeatedly and quickly with the antennae (antennal clashing), while the dominated (δ) takes on the characteristic position of akinesis, which often goes on after the end of the antennal clashing. In Table 1 are presented some approaches of different types, in the order of increasing hostility of Δ, from her complete tolerance of the partner to the maximum of hostility. The behavior patterns assigned the same number happen with approximately equal frequency, but they are not the only ones possible.[4]

In a series of contacts between two individuals, providing certain conditions are constant, it is always the same individual that dominates.[5]

[4] For a fuller explanation of the subject see my study to be published in Behaviour: Internat. Jour. Comp. Ethology (Leiden).

[5] Some of these activities (antennal clashing or caresses, solicitation of nectar, walking of one wasp up to another) have been observed by Rau (1938, p.

If A dominates B, the same events happen during all the successive approaches. With reference to analogous phenomena in the birds, we are entitled to speak of a hierarchy of the "peck-right" type.

This rule of the stability of reciprocal behavior, from which consequently springs a tendency toward hierarchy stability, has rather rare limitations of two types.

1. In a certain contact it sometimes occurs that δ shows an initial resistance and attempts to assume the behavior of the dominant; but soon δ yields and eventually acts as dominated, taking on the position of akinesis, for instance. With reference, again, to the vertebrates, we can compare this type of behavior to the "dominance with resistance" noticed by Uhrich (1938) in white mice.

2. In other cases, though very rarely, a dominance of the "peck-dominance" type is observed, that is to say, an individual, A generally > B, sometimes can be dominated. In this particular case we call the individual "dominant" that dominates during the majority of their contacts.

TABLE 1

BEHAVIOR OF THE DOMINANT AND OF THE DOMINATED INDIVIDUAL

Δ (Dominant)	δ (Dominated)
1. Gives liquid to δ, with erect head, without antennal clashing	1. Stretching her head toward Δ, trills the mouth region of Δ from below
2. Moves toward δ	2. Avoids the approach by turning sharply aside when in proximity of Δ
3. Antennal clashing; strikes or touches lightly the head of the partner, the eyes generally, with alternating motion; more rarely strikes the thorax and the abdomen	3. Position of akinesis (body adherent to the nest, bent head, antennae and legs in flexion); otherwise, between the mandibles shows a regurgitated liquid drop ("offer"), which is sucked by Δ; after these reactions, exit from the nest often occurs
4. Bites δ on the genae, on the eyes, on the thorax, and, less frequently, on the abdomen	4. Akinesis or offer (see No. 3)
5. Bites violently, clasps δ with legs, spreads the wings sharply in an attempt to pull δ out of the nest; sometimes stabs or attempts to stab	5. Akinesis or offer of regurgitated liquid, which Δ does not suck generally; runs away quickly (taking shelter behind the honeycomb or in the cells) or goes out of the nest

At the end, the dominant, growing old or, generally, becoming weak, can be overpowered and dominated by the same individuals that she had dominated previously: therefore a *social regression* of some individuals is possible, often preceded by a brief period during which furious struggles are engaged in between the regressing wasp and those that have become equivalent.

Equivalence.—In addition to the above-mentioned cases, contacts are observed during which two individuals appear equivalent in their behavior and absolutely intolerant of one another.

233; 1940, p. 618, and elsewhere) in various species of American *Polistes*. Though Rau has seen (like Heldmann [1936] in the European *Polistes*) that "the reciprocal relations between three founding females seemed to be practically the same as between one queen and two workers," he pays no attention to the following essential fact, which demonstrates the existence of a dominance order, that is, that in an approach between two individuals it is always the same one that dominates or is dominated. Therefore, it is clear that Rau cannot speak of "hierarchy," though the hierarchy is based just on the reciprocal relations correctly observed by him. Rau also interprets these behavior patterns as forms of social communication, without putting in the proper evidence concerning the competition which they indicate.

They fight violently and strike one another with the prothoracic feet, the antennae, and the stings. This happens mainly when certain individuals of different nests meet or, as already mentioned, in social regressions.

TABLE 2

A DIAGRAMMATIC SOCIAL HIERARCHY OBSERVED IN A NEST OF *P. gallicus* (L.) DURING THE POLYGYNIC ASSOCIATION (NEST I: MARCH 23–APRIL 6, 1945)

Individual Numbered	Number Dominated	Individuals Dominated					
37	5	6	13	...	1	8	3
3	4	6	13	...	1	8	...
8	4	6	13	4	1	...	...
1	3	6	13	4	...	...	...
4	2	6	13	...	...	...	...
13	1	6	....	...	...	...	...
6	0	...	....	...	...	...	...

THE LINEAR ORDER OF THE HIERARCHY

A rigorously linear hierarchy often results from all the possible contacts between the inhabitants of the same nest (females or workers) (Tables 2 and 3):

$$\alpha > \beta, \gamma, \ldots . \omega$$
$$\beta > \gamma, \ldots . \omega$$
$$\gamma > \ldots . \omega$$
$$\omega > \text{none}$$

Briefly, we call the place occupied in the dominance scale "social status." Only the females and the workers seem to participate in the linear organization. All the males behave as dominated in their relations with the workers, which often and actively persecute them. On the other hand, as far as the observed contacts between males are concerned, it is not possible to derive a clear meaning for them.

In the *Polistes* society the individual α corresponds to the so-called "queen" of the traditional nomenclature; but, when the queen disappears, the α-place can be occupied by one of the workers that becomes fertile (*substitution female*). During the polygynic association, α is the leader.

Also the rule of the linear organization has a limitation. Before the spring foundation of the new colony, the linear organization is complicated by the appearance of "triangles" between the females that meet near the maternal nest. This fact demonstrates the coexistence of many factors, which simultaneously influence the result of the contacts. The same phenomenon is also observed during the period of the greatest social activity (in July and August), when the colony is numerous because of the presence of many workers. In all the other periods (that is to say, during the polygynic association and soon after the eclosion of the first workers) the organization is typically linear.

THE FREQUENCY OF DOMINANCE AND PASSIVENESS IN THE INDIVIDUALS OF THE HIERARCHY

The total frequency of dominance of a certain individual (that is, the number of times an individual acts as dominant over all the other inhabitants of the nest) is dependent on its social status. Also, as to the mean frequency of dominance

TABLE 3

A SOCIAL ORDER OBSERVED IN A NEST OF *P. gallicus* (L.) AFTER THE DEATH OF THE QUEEN (NEST I: JUNE 6–12, 1945)

Workers in Order of Eclosion	Number Dominated	Individuals Dominated								
1	9	15	12	11	8	7	5	4	3	2
2	8	15	12	11	8	7	5	4	3	...
3	7	15	12	11	8	7	5	4	3	...
4	4	...	...	11	8	7	5	...	...	...
5	5	...	...	11	8	7	...	4?	...	2?
7	3	15	...	11	8	...	...	...	...	...
8	2	15	...	11	...	...	...	...	...	...
11	0	...	...	...	...	...	...	...	...	...
12	0	...	...	...	...	...	...	...	...	...
15	0	...	...	...	...	...	...	...	...	...

(total frequency: number of individuals dominated), generally the higher it is, the higher is the social status (Table 4).

The distribution of the dominance frequency of an individual upon every one of its subordinates depends, with satisfactory approximation, on the social status of the subordinates, that is, an individual dominates the one that occupies the place immediately below it in the social scale with higher frequency, while she dominates all the others with decreasing frequency (Table 4 and Fig. 1).

Because of the two preceding rules, it is clearly evident that the most passiveness is shown by the wasps which are nearer in the order to the α; ω, though theoretically dominated by all the partners, actually is dominated infrequently.

The frequency of passiveness is influenced by the age of the individuals, because the probability of one wasp's meeting the others is dependent on its age; in fact, on the first days after the eclosion, all the wasps generally remain behind the comb (*Jugendruhe* of Steiner).

VARIATIONS IN THE SOCIAL STATUS

The variations in the social status are to be ascribed to three different causes: death, social regression of some components of the colony, and, the addition of new individuals.

TABLE 4

FREQUENCY OF DOMINANCE AND PASSIVENESS OF INDIVIDUALS OF A *P. gallicus* NEST DURING SEVEN OBSERVATION DAYS AFTER DEATH OF QUEEN (NEST I, 1945: OBSERVATIONS JUNE 6–12, FOR A TOTAL OF 8 HOURS)

Boldface numbers indicate individual wasps. Other figures indicate number of times an individual was observed to be dominant over its mates. Worker No. 1 was the substitution female. With the omitted numbers in the order of eclosion were marked five males.

Workers in Order of Eclosion	Workers in Order of Eclosion										Total Frequency of Dominance	Mean Frequency of dominance
	1	2	3	4	5	7	8	11	12	15		
1		60	47	21	17	9	4	6	2	2	168	18.6
2	.....		22	15	4	6	1	3	1	3	55	6.8
3	.....	.....		9	15	20	2	4	4	1	55	7.8
4	.....	.....	.....		4	1	3	1	.....	.....	9	2.2
5	.....	1?	.....	1?		3	4	3	.....	.....	12	2.4
7	.....	.....	.....	.....	.....	.....	1	3	.....	2	6	2.0
8	.....	.....	.....	.....	.....	.....	.....	2	.....	1	3	1.5
11	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	0	.....
12	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	0	.....
15	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	0	.....
Total frequency of passiveness	0	61	69	46	40	39	15	22	7	9	308	.....

As to the first and the second cases, the individual immediately lower in the dominance scale takes the place of the dead or regressed wasp. So, during the polygynic association, the successive deaths of several wasps of the initial hierarchy can determine the acquisition of the α-status by one of the last partners (Fig. 2). After the eclosion of the workers and the elimination of the auxiliary females, if the queen dies, it is immediately followed by the worker that occupies the β-position; soon this worker behaves as did the queen, dominates its own mates, mainly on the first days, with singular

frequency, and, in very short time, is able to lay eggs.

In the case of social regression, it is not yet clear whether the regressed individual always descends only one step of the dominance scale; observations seem to justify the conclusion that, after the loss of the previous position, the regressed individuals quickly go down to the lower places of the social hierarchy.

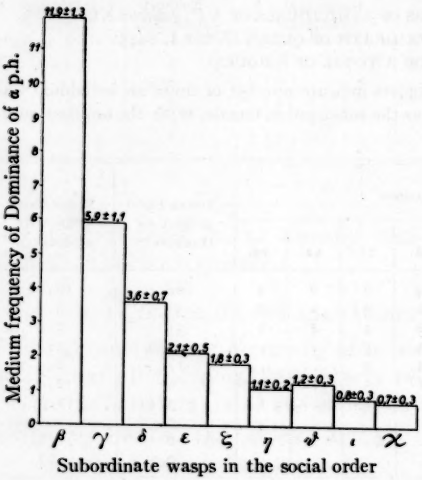

FIG. 1.—Medium frequency of dominance of α over its subordinates. The numerical value of the frequency has been obtained by taking the average between the frequencies observed during 20 observations over Nests I, II, and III (May and June, 1945). The observations concerning each nest lasted 1 hour.

From other preliminary observations, which, however, are to be extended and confirmed, it appears that the regression commonly happens in those individuals which suffer the greatest dominance by α, that is, in the β or γ individuals.

The addition of new units in the colony rarely occurs after the eclosion of the workers; on the contrary, it is frequent during the polygynic association. As I have demonstrated elsewhere (Pardi, 1947), when an addition takes place the new individual usually is not perfectly equivalent in dominance to any one of the association components. When individuals are perfectly equivalent, furious fights take place between the equivalent individuals, and their coexistence in one colony is impossible. For instance, the incompatibility between two α wasps and also between two β's is absolute. When nonequivalence in social status is real, the new individual comes into the hierarchy and occupies a position which depends on factors that will be made clear below. It is possible that the newcomer may overpower all the others and occupy the α-position or take no status whatever until it reaches the ω-position. In all these cases we observe variations (that may be easily foreseen) in the social status of the individuals that had already co-operated in the association (Fig. 2).

DOMINANCE FACTORS

Size and other physiological conditions of the ovaries.—The dissection of the ovaries demonstrates the existence of a relation between ovarian size and the position in the social hierarchy. An index in the ovarian size can be obtained by measuring the length of the proximal eggs of each of the six ovarioles and taking the average; it is superfluous to warn that such a measure rather imperfectly expresses the real individual differences in the physiological condition of the ovary. However, it is clear that in the contact between any two individuals the dominant is generally the one with the higher ovarian index and is also the individual with the ovary of greater size.

It seems probable that the ovaries have an influence, perhaps by means of internal secretion, on determining the form of behavior. If such a relation is confirmed definitively, the analogy with the vertebrates will be much closer.

The hierarchy in the spring polygynic association is established in accordance

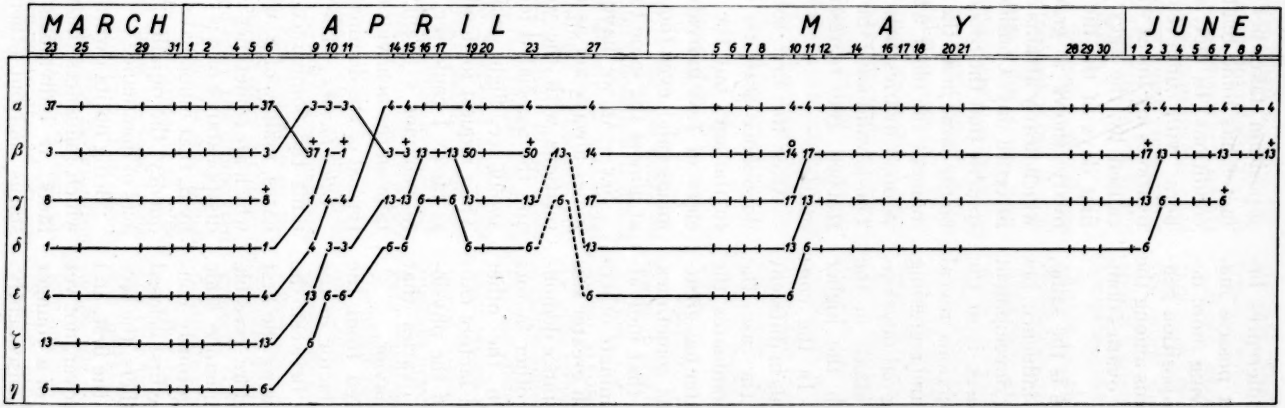

FIG. 2.—Variations of the social status in a polygynic nest of *P. gallicus* (Nest I, 1945) from March to June (end of polygynic association). At the top are the observation days. A little cross indicates death, a circle indicates the transfer of a female to another nest. The No. 4 female comes up from position ε to position α, after the death and the regression of its mates in higher social status.

with the small ovarian differences between the individuals that possess mature eggs. The females having more developed ovaries take the α-position generally. Just the same happens among the workers: those with greater ovaries dominate generally.

If the size of the ovaries is the same, other ovarian conditions influence behavior; the stage of the egg development of two individuals that meet is of the greatest importance. Dissections reveal that wasps have ascending and regressing phases in the ovarian imaginal development. Between two individuals in the same phase, the one with the higher ovarian index dominates. In the contacts between two individuals in different phases, in the majority of the cases the one in ascendant phase dominates the other, particularly if the latter has greater ovarian size. It happens sometimes, though much more rarely, that individuals in regressing phase dominate others in ascending phase and with greater size of the ovaries. This circumstance demonstrates that there are still other factors influencing dominance. On the other hand, the above-mentioned factors cannot give a complete idea of the physiological conditions of the ovaries that could have an effect on behavior.

Age.—The age (computed from the eclosion) is an important factor in the formation of the hierarchy between the workers; immediately following the first eclosions and therefore before possible social regressions, the dominance scale between the workers is rigorously connected with the age: i.e., the first eclosed worker dominates all the others, the second all the others less than the first, and so on. Very rare (and, moreover, not yet reported) is the dominance of a younger worker over an older one. Consequently, if the queen is missing during this period, the oldest worker, that is, the one in β-position, takes her place. Perhaps this factor has some relation to the ovarian conditions. In fact, all the workers are born with undeveloped ovaries (the ovarioles are linear without the swellings caused by the eggs), although, on the first days of the life of the imago, the ovary shows a gradual development, which nearly attains its maximum on the fifteenth day. Considering that it is reasonable that the process of increases may be the same in all the workers, at a given moment the older wasps have more developed ovaries than the younger ones. This is valid until the older workers have reached the regressing phase of the ovaries.

After the first social regression, the dominance scale no longer corresponds to the age; but the rule by which the elder of two individuals dominates remains valid, even though they are eclosed at almost the same time.

Sex.—As we have already said, the males always act as dominated in their relations with the workers, the queen, and the associated females (reactions of avoidance, flight, akinesis, and so on). Their request for food from the workers, as far as I could see, happens pursuant to behavior 1 (Table 1), that is, they take on the behavior of request, which is typical of the dominated individuals.

Other factors.—The presence of α maintains the dominance over the subordinates, mainly of β, within certain limits which are sometimes very restricted. The disappearance of α is perceived very soon by β; that immediately increases the frequency, the rapidity, and the fury of its acts of dominance.

Being on its own nest is also a factor which influences the result of some contacts. If individuals of known social status confront others of known social status, belonging to a different nest, it has been noted that an individual, domi-

nated by some wasps when it is on the foreign nest, is able to dominate and to drive out the same wasps when on its own nest. The phenomenon recalls once more the facts ascertained concerning the vertebrates and the relation between dominance and territory.

The individual α, growing old, shows a lower dominance frequency but is able to maintain supremacy for some time. Probably when the process of growing old is revealed by a clear change in behavior, the individual α is attacked, dominated, and, not rarely, violently driven out of the nest. Weakening for other reasons, such as a casual injury or some operation performed as an experiment (the ablation of the antennae, for instance), has given the same results.

RESULTS OF THE DOMINANCE ORDER

Place on the nest.—The rest place on the nest is in relation (though it is not a strictly rigorous relation) to the social status of the individuals. Generally, the individuals of higher position in the scale stay on the anterior face of the comb for a longer time. The regressed and older workers, in the great majority of the cases, rest behind the comb, and, if persecuted, hide themselves in the cells like the males.

Trophic advantage.—The dominance behavior is connected with an advantage of trophic nature. The contacts of the type 3 (Table 1), which show a transfer of regurgitated food from the dominated to the dominant individual are much more frequent than the contacts of type 1, which sometimes show the food transfer in the opposite direction. The dominance system regulates the distribution of the liquid foods regurgitated from the social stomach. The regurgitated liquids are of external source (sugared vegetable liquids), or they are of social source (secretions obtained from the larvae during trophallaxis).

Work distribution.—A definite relation exists between the social status and the work distribution. This is clearly shown during the period of polygynic association. After the reactions of type 3 (Table 1), the exit of the dominated individuals from the nest often occurs. Therefore, absence from the nest and the work connected with gathering of nutritive material and wood pulp, building, etc., is much more frequent among the individuals of lower social status. On the other hand, the females of higher position in the order tend to assume certain activities which do not require absence from the nest (laying eggs, inspection of the cells, resting, and so on) (Fig. 3). The α is inclined to specialize in laying eggs.

Fertility and sterility.—As we have seen, in the beginning of the spring association, the dominance order is established according to the ovarian status; i.e., the individuals with better-developed ovaries generally are more dominating. On the other hand, the trophic advantage, which is connected with a very frequent dominance function, influences the ovarian development; as the α-individual gets more and more developed ovaries, the other associated females have a tendency toward regression. At the end of the polygynic association, for instance, α possesses very well-developed ovaries; those of the auxiliary females are more regressed, the lower their position in the dominance scale (Fig. 4)

After the eclosion of the workers and the elimination of the auxiliary females, the worker which is in the β-position when the queen dies becomes α and, in few days, is able to lay eggs. Successively, also, other workers (always of higher social status) can become fertile. Elsewhere (1946) I tried to find the minimum

in the social scale sufficient for the acquisition of fertility, but definitive researches concerning this point have not yet been performed.

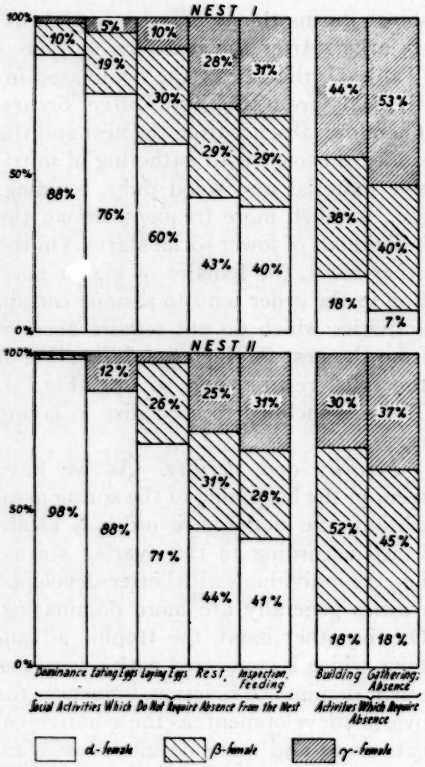

FIG. 3.—Work distribution among the association founding females of two trigynic nests of *P. gallicus* (Nest I and II, 1942). Observations from March 13 to July 1: total duration, 142 hours. Percentages indicate the part played by the single wasps in every social activity, with 100 indicating the number of times the given activity was observed on the nest. A few β–γ contacts with no decisive results are registered as γ-dominance.

On the other hand, it is not improbable that the continuous subtraction of nutritive liquids among the more frequently dominated wasps causes a state of trophic deficiency to which, in part at least, the ovarian regression might be attributed.

There is also another way through which dominance can influence the fertility and the sterility of the components of the colony: the work distribution. This point is supported by the observation that the auxiliary females of lower position in the polygynic association, which work hard and are compelled to give regurgitated liquid less frequently than the auxiliaries nearer to the leader in social status, show at the end of the polygynic association a much more conspicuous ovarian regression. In my opinion, the fact cannot be explained alone by the initial small differences in size of the ovaries in the associated females, but it should be interpreted as a proper "castration for work," caused by energy consumption dependent on intense work.

However, it is certain that both these factors (work and the giving-up of regurgitated liquids) are connected directly with the dominance system.

It is particularly interesting, in regard to the same point, to remember that a relation between dominance and laying eggs exists also among the vertebrates. According to Sanctuary (quotation from Allee, 1945*b*): "The hens in the lower part of the social order of the flock laid fewer eggs than those with higher social rating, but . . . . if segregated away from their more dominant flock mates their rate of egg production increased decidedly."

CONSIDERATIONS

The conclusion which we have arrived at, that is, the existence of a strict relation between the dominance system and the work distribution within the society, suggests some considerations of comparative nature.

Allee (1940), starting from the idea of the unconscious co-operation, the importance of which in the social phenomena of the animal kingdom had already

been recognized by Espinas (1878) and by Wheeler (1923), notes that,

> given an underlying stratum of co-operation, competition, by establishing degrees of dominance and subordination, whether in the ecological or human sense, may furnish a basis for more effective community co-operation. Such organized communities may then compete with each other and the less fit co-operating systems may be weeded out to the general advantage of the survivors at least.

with other social Hymenoptera like bees and ants, the work distribution, mainly during the polygynic association, appears as a consequence of the dominance system, while the dominance mentioned is the result of intrasocial competition. Undoubtedly, considering the association as a whole, the work distribution represents an advantage for the society. Therefore, it is reasonable to think that

FIG. 4.—Ovaries of *P. gallicus* females at the end of the polygynic association (June). *1–3:* ovaries of three females of Nest I; *4–5:* ovaries of two females of Nest II. *1* and *4:* α-females with well-developed ovaries and mature eggs; *2* and *5:* β-females with regressed ovaries; *3:* γ-female with great regression.

An experimental confirmation of this principle was given recently by Allee himself (in collaboration with Guhl):

> There is some justification for thinking that these laboratory findings are indicative of similar relations in the wild. If so, individual-against-individual competition, such as brings about the peck-order type of social organization of flocks of hens, may serve to build a co-operative social unit better fitted to compete or to co-operate with other flocks at the group level than are socially unorganized groups [Guhl and Allee, 1944, p. 346].

Now in *Polistes*, which in the characteristics of its organization is a rather primitive social insect in comparison in the primordial origins of social phenomena among the insects, associations wherein competition between the individuals with the above-mentioned useful consequences was established had survival values in nature. In this sense we find in *Polistes* a stage much more advanced than among the birds of the process which, according to Allee, leads from mechanical and unconscious co-operation to the establishment of true organized societies. It is obvious that the competition must not be too severe, otherwise association is not possible.

It should be of great interest to see if

among other social insects there are phenomena of competition like those ascertained in *Polistes*. Among the Bombini (Apidae), which, because of their primitive social organization, represent the stage corresponding to the Polistini in the series of the social bees, it is probable that phenomena of competition occur.

The existence of phenomena of dominance among the ants is more problematic. Schjelderup-Ebbe reports to me that he has observed a sort of "despotism" in *Formica rufa*.[6] On the other hand, from the classic descriptions of the life of the ants (Gould, Huber, Forel, Wasmann, etc.; see Wheeler, 1923) it seems reasonable to deduce the existence of competition not only between different species (*dulosis*) but also between individuals of the same species and of the same colony. I am now undertaking researches on the problem.

As far as the other invertebrates are concerned, the beginning of a social hierarchy based on strength is found, according to Allee and Douglis (1945) in the hermit crab, *Pagurus longicarpus* Say.

SUMMARY

A social hierarchy quite similar in general form and meaning to those described for many vertebrates exists in *P. gallicus* (L.) (Hymenoptera-Vespidae).

Two individuals of *P. gallicus* rarely show equivalent and tolerant behavior; generally they fight each other furiously (equivalence and intolerance), or one of the two takes on the behavior of a dominant, the other acts as dominated (non-equivalence). The males generally present dominated behavior.

In a series of contacts between two individuals, if certain conditions are permanent, it is always the same individual that dominates.

From all the possible contacts between the females of the same nest, a sort of social order results, a "dominance scale."

During the association between the wintering females which in the spring found a nest together (polygynic association), the dominance order is rigorously linear.

The α is the leader or queen of the nest, and it lays eggs always; the other females lay eggs only at the beginning of the association, then their ovaries regress; and after the eclosion of the workers (in June) they are eliminated.

The workers are always subordinate to the queen and also to some auxiliary females. A linear dominance order, dependent on age, exists among the first workers. Successively, certain regressions in social position happen, so that the order no longer corresponds exactly to age.

If the queen is missing or earlier becomes regressive, it is the older worker (the one in the β-position) that occupies her place. In the same way, if the queen disappears later, she is followed by a worker in the β-position. This worker, occupying the queen's place, is able to lay eggs in a short time. Also some other workers in the higher part of the social order may become fertile.

During the polygynic association and after the eclosion of the workers, each individual shows the maximum frequency of dominance toward the one immediately below it in social position.

The following factors influence dominance: the condition of the ovaries (in the contacts between two individuals, it is the one with more developed ovaries that dominates); age, perhaps in strict relation with ovarian development; sex; growing old or other debilitation; some psychological factors.

[6] *In litteris* (April 2, 1947), and in the journal *Nationen*, June 9, 1944.

The different places occupied on the nest is a fact connected (though not rigorously) with the system of dominance.

Dominance causes a trophic advantage, considering that the case of a dominant female being fed by a dominated one is much more frequent than is the opposite condition.

A relation between dominance and work distribution also exists: i.e., the individuals of lower social status undertake the greater part of the external work, while the work on the nest is done mainly by the wasps in higher position. The α specializes in laying eggs.

Among the females that are somewhat more active than passive in their behavior, social domination brings a further ovarian development and fertility as a consequence of the action of the connected advantage of a trophic nature. The system of dominance influences the condition of sterility, which, it seems, is to be attributed, in part at least, to a sort of castration for work in the dominated individuals of lower social status. In *P. gallicus* the production of sterile and fertile forms is an indirect consequence of the dominance system, and it is independent of the nutrition received during the larval life.

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THE SEXUAL BEHAVIOR OF INTERSEXUAL DOMESTIC FOWL[1]

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IT IS now widely believed that endocrine conditions greatly influence, or even control, the behavior of many animals. By utilizing proper experimental techniques it is possible to observe the effect of certain hormones and thereby study their influence on some of the components of behavior. The stereotypy and vigor of sexual behavior, as well as the relative simplicity of operational and injectional techniques, provide exceptional means for analyzing behavior patterns. Surgical removal of endocrine organs and hormonal replacements make possible the segregation of some of the constituents of sexual behavior.

Birds in general, and especially the domestic fowl, are particularly suitable for a study of behavior problems, since they behave in a fairly consistent manner and since the fowl, unlike most birds, does not require preliminary pairing before coition will take place. Unfortunately, very little is known about the behavior of the ancestral *Gallus bankiva* in this respect.

This investigation concerns the sexual behavior of intersexual roosters. An attempt is made to segregate the various components of sexual behavior and to analyze their occurrence and origin. The viewpoint throughout is psychoanalytical, as exemplified in recent ornithological research. In addition to a detailed consideration of sexual behavior, certain observations concerning peck-order pertinent to the problems under discussion are recorded. Two preliminary statements have preceded the present definitive report (Domm and Davis, 1941, 1942), and the influence of hormones on sexual behavior (Davis and Domm, 1943) has been studied.

Studies on the domestic fowl from the viewpoint considered in this article have not been previously attempted. Although Skard (1936), in extensive observations on two flocks of hens, investigated the sexual needs of hens, she did not examine the endocrine aspects. On the basis of observations on the behavior of two cocks and about thirty hens, she described sexual behavior in some detail. In addition, several investigators have mentioned sexual behavior incidentally in connection with other problems. In his investigations on the effects of ovariotomy, Domm (1927) recorded observations on the sexual behavior of poulards. Allee and co-workers (1939, 1940*a*, 1940*b*), while investigating peck-order or social hierarchy of chickens, recorded incidental observations on sexual behavior, while Guhl, Collias, and Allee later (1945) made a study of mating behavior in

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The essential data presented in this paper were summarized in reports given at the meetings of the American Society of Zoölogists held in Dallas, Texas, in December, 1941, and of the American Association of Anatomists held in New York City in April, 1942.

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White Leghorns. Investigators interested in more practical aspects have approached the problem from a different viewpoint, as indicated by Parker, McKenzie, and Kempster (1939), who studied frequency of copulations in roosters and found that, although more copulations occurred in the afternoon, sperm was less abundant at this time. Other investigators have analyzed sexual behavior from the psychological viewpoint. The pioneer studies of Heinroth (1910) were followed by the important ornithological work of Lorenz (1935, 1937), Tinbergen (1939*a*), and many others. In the purely experimental field the investigation of Carpenter (1932, 1933) on pigeons provides information concerning the endocrine basis of sexual behavior. He observed that copulation and billing were reduced in frequency but not entirely eliminated by complete castration.

MATERIALS AND METHODS

The Brown Leghorn breed of domestic fowl was used in this investigation. The birds were intersexual males hatched from eggs that had received a single injection of estrogen[3] on or before the fourth day of incubation. At the time of the experiments the birds were 22–24 months old. The sexual organs were variously modified. In brief, the gonads were ovotestes, containing various proportions of ovarian and testicular tissue; commonly a left and frequently also a right oviduct were present. Parts or all of one or both oviducts were sometimes distended with a clear watery fluid, resulting in large abdominal tumors (Domm, 1939, 1940*a*, *b*).

The majority of the males showed varying degrees of femininity in plumage and for purposes of the experiment were divided into four arbitrary classes on the basis of plumage pattern. A description of the characteristics of each class follows.

Class I[4].—These birds have normal or nearly normal masculine plumage. The hackle is long and Mars Orange to Mars Yellow; the back feathers are masculine; some of the saddle feathers are shorter than normal and poorly colored. The wing bar, although masculine, is reduced in size, while the tail is typically masculine. Breast, ventral, lateral, and fluff feathers are male. The comb is large and erect, and spurs are present. In general appearance the bird is a normal male.

Class II.—The birds of this group show somewhat less well-developed masculine characters. The hackle is Burnt Sienna to Mars Yellow and shorter than normal. The back feathers are Brussels Brown, and the wing bar is almost absent. The saddle is short and colored Brussels Brown but is not definitely feminine. The breast has some of the Auburn, feminine feathers of the female, but is mostly Black. The comb is large and usually erect or slightly loppy. Spurs are well developed. In general appearance the bird is obviously feminized.

Class III.—The saddle of these birds is short and colored Mars Yellow. The masculine wing bar is absent. The tail, however, is somewhat masculine, and the breast possesses some Black feathers, although it is mostly Auburn. The comb is large and may be erect but is usually loppy. Spurs are present. In general ap-

[3] The estrogens employed were alpha-estradiol benzoate, theelin, and diethylstilbestrol. Grateful acknowledgment is made to Dr. Erwin Schwenk, of the Schering Corporation, for the alpha-estradiol benzoate (Progynon-B); to Dr. Oliver Kamm, of Parke, Davis and Company for the theelin; and to Dr. J. A. Morrell, of Squibb and Sons, for the diethylstilbestrol used in these experiments.

[4] The capitalized names of colors are those given in Robert Ridgway, *Color Standards and Color Nomenclature* (Washington, D.C.: The author, 1912).

pearance this bird shows only traces of masculine plumage characters.

Class IV.—These birds have feminine hackle, back, saddle, and breast feathers. Wing feathers are also female. The tail sickles may be slightly masculine. The comb is frequently smaller than that of the male and usually loppy. Spurs are present. In general appearance these individuals are nearly indistinguishable from females.

The birds were housed in pens in the poultry house adjacent to the laboratory. The pens were 18 feet long and 7 feet wide and were connected with outside sun porches with wire bottom and sides, which were 5 feet square and 4 feet high. The cement floor of the pens was covered with a litter of wood shavings, changed weekly. Trap nests were provided, and a roost with dropping board was located in one corner. The food consisted of a standard mash and mixed-grain ration prepared according to a formula recommended by the University of Illinois Poultry Experiment Station. Dry mash, meat scraps, charcoal, grit, oyster shells, and water were in the pen at all times, while a wet mash was fed in the morning and mixed grain in the afternoon.

The number of birds in each pen was between five and nine and usually included intersexual males from each of the four plumage classes, as well as three or four females. The same birds had been in the pens for several months before observations began. Although several birds died during the experimental period, thus reducing the number of birds in each pen, no new birds were added to any of the groups except near the end of the experiments. Each bird carried numbered and colored celluloid bands on the legs for individual identification.

The experimental techniques consisted of various methods of introducing the intersexual male to a female, to a normal rooster, or to a stuffed dummy hen. In order to increase the tendency to copulate of males to be tested, they were placed in isolation cages for varying periods usually ranging from 12 hours to several days.

A preliminary series of trials indicated that the intersexual males would react to a female introduced into their pens. After several procedures had been tested, it was decided that the following techniques best indicated the sexual behavior of the experimental birds: (1) Introducing each intersexual bird into an unfamiliar pen in which there was a female. (2) Introducing a female into the pen of each intersexual male after having removed all other birds. (3) Removing the females from the pens in the afternoon, and the following morning introducing the males serially (i.e., one after another) to the females. Thus the males were together in their own pen but isolated from the females of their pen. It should be noted that in this case the females were known and recognized as individuals by the males. (4) Removing the females for 2 days and introducing them as in step (3). (5) Removing the males at 11:00 A.M. on one day and returning them serially to their own pens and female penmates at 4:00 P.M. on the second day. (6) In a few instances the males were left overnight in a strange pen with a strange virgin female. The eggs from these hens were saved and incubated. This experiment was designed primarily to test the fertility of intersexual males.

A stuffed dummy hen, mounted in the squatting copulatory position, was used in the experiments in addition to live birds, since the hen cannot always be relied upon to squat on the approach of the rooster. This method has been used

successfully with wild birds (Friedmann, 1929; Allen, 1934; Chapman, 1935; Noble and Vogt, 1935; Lack, 1939). Although the dummy became battered and worn and was repaired from time to time during the course of the experiments, its use eliminated one of the variables (the female) in behavioristic experiments by providing a constant object-stimulus. Preliminary experiments had shown that a normal rooster will try to copulate with even a dummy cock mounted in the normal standing posture; hence it was not surprising to find that the males readily copulated with the stuffed female. The intersexual males were tested with the dummy in exactly the same manner as with normal females. Sometimes the test with the dummy hen preceded that with normal hens and sometimes it followed.

In order to provide additional information concerning the sexual activity of these intersexual birds, the eggs from normal hens mated with them were saved and incubated to determine their fertility. In such cases, obviously, no hen was used with more than one male. Furthermore, the eggs laid daily in all pens were routinely collected and incubated. From these latter data it was possible to determine which pens had fertile males. Then to determine which male was fertile, procedure 6, outlined above, was applied to each of the males of that pen.

The experiments were begun during the last week of March, 1941, and continued until the end of July. The most intensive work was done in April and May. A total of 40 intersexual males and 32 females from similarly treated eggs were observed. Normal roosters and normal hens were used in the experiments. Each intersexual male was tested approximately ten times in experimental situations.

PECK-ORDER OF INTERSEXUAL BIRDS

During this study on the sexual behavior of intersexual roosters, many incidental observations on the peck-order were obtained. These data confirm the principles already worked out (Schjelderup-Ebbe, 1922, 1935; Masure and Allee, 1934; and Allee, 1947) and, in addition, present certain interesting facts concerning the factors which determine the position of the individual. The peck-order in each pen was determined by watching the pecking of the birds at the feeding trough. These birds had been together in the same pens for about a year and therefore the peck-order was well established.

The birds in each pen had a definite social hierarchy whereby a superior individual could peck an inferior with impunity. Tables 1–4 show the behavior of all males in the four plumage classes. In the column labeled "Rank" is given the relative position of each bird in the social hierarchy. The first figure represents the position measured from the top and the second is ranked from the bottom. Thus, for example, No. 1509P (Table 1) was the top bird in his pen, and there were two birds below him. Again, No. 1522T (Table 1) was the middle bird in a pen containing three male birds. Again, No. 1728U (Table 1) had one bird above him and three below him. Birds possessing the most masculine plumage (Class I) were generally at the top, while those possessing feminine plumage (Class IV) were as a rule at the bottom of the social hierarchy. Although the rank of an individual appears to depend upon several factors, the hormonal factor is of primary importance. A bird may advance in rank as a result of the injection of testosterone (Guhl, 1942). Accordingly, the intersexual birds usually assumed a rank re-

lated to their gonadal condition as represented by plumage. These birds all belonged to the same breed, hence heredity had a negligible influence on peck-order, contrary to the situation in flocks of mixed heredity (Fennell, 1945).

Another important factor in peck-order is the previous history of the individuals. A bird introduced into a strange pen of birds does not assume its rightful rank for days or even weeks because the occupants familiar with the pen and their flock mates have a great advantage. Still another factor, namely, the effect of the bird's state of well-being, is important (Collias, 1943). As recorded elsewhere (Domm, 1940*a*), these birds commonly reveal a pathological condition of the oviducts as a result of embryonic hormone treatment. Several of the males had greatly distended oviducts, weighing in some cases as much as 500 gm. Such individuals were regularly lower in the peck-order than would be expected from a consideration of the plumage displayed. For example, No. 1550B possessed the plumage of Class I individuals and had an enlarged abdomen, caused by an oviducal tumor that compelled him to waddle like a duck. He was at the bottom of the peck-order in his pen and died a month after the experiments ended. Apparently, his physical handicap diminished his fighting ability. In only one case was a male below the females. No. 1605W became lame and for a time was below several females in his pen but subsequently recovered his original position in the peck-order.

It should be noted that the intersexual males and the females in each pen formed one continuous series in peck-order. Normal males and females, on the contrary, form two distinct groups because the males do not peck the females. The intersexual roosters being partially feminized, formed an intergrading series from male to female.

DESCRIPTION OF SEXUAL BEHAVIOR OF NORMAL BIRDS

A description of the sexual behavior of normal birds is a prerequisite for an understanding of the behavior of abnormal individuals. This section gives a detailed behavioristic description of the action of males and females in the pens under observation. While it is true that these birds under the artificial conditions of domestication may behave very differently from the wild ancestral jungle fowl, nevertheless, for present purposes, their actions may be considered normal.

BEHAVIOR OF MALE TOWARD FEMALE

When a female is introduced into a pen with a rooster, he at once pursues and seeks to copulate. She may follow one of two courses. If she is receptive, she will squat with the wings slightly raised and held away from the body, the tail raised into a vertical position, the feathers appressed to the body, and the neck, head, and bill stretched horizontally about an inch above the floor. The rooster usually mounts on the run from either side, grabs the comb or not infrequently the neck feathers in his beak, and, while fluttering his wings, appresses his cloaca to that of the female several times in rapid succession and dismounts. Upon dismounting, the rooster almost invariably immediately ruffles his feathers and vigorously shakes his body. He may then waltz and half-circle the hen and, if she is still squatting, will frequently mount and repeat copulation. If, on the contrary, the female is not receptive, she will avoid the male if possible. Even so, he may be able at times to catch her and force copulation, or in extreme cases she may succeed in evading him by flying to

the roost. If the female persists in avoiding the rooster, he usually gives up and leaves her alone.

BEHAVIOR OF MALE TOWARD DUMMY FEMALE

When exposed to the stuffed dummy hen, males usually approached it on the run and copulated in the normal manner, in spite of the fact that the dummy was not in a perfect squatting pose and even after it had become bedraggled in appearance. The fact that the neck feathers of the dummy eventually were lost, the wings shoved out of place, and the tail broken and held in place with rubber bands did not seem to modify the behavior of the male. The tendency to copulate of the normal male, especially if he had been isolated from females for a day or more, was so great that the dummy was an adequate stimulus to copulation.

BEHAVIOR OF MALE TOWARD MALE

When two unacquainted roosters are placed in the same pen, they face each other, lower their heads, and ruffle the neck hackle feathers. The birds stare at each other, breathe vigorously, and jockey for a favorable chance to strike. After a short interval the birds jump at each other, grabbing for each other's comb and striking viciously with their spurs. This performance is continued with fluctuating vigor until one of the pair admits defeat by running away. The victor pursues while inflicting vicious blows with beak and spurs to the head and body of the vanquished. This usually continues for a period, with gradually diminishing intensity, unless the latter succeeds in evading his adversary. This fighting is not a part of sexual behavior as such but is probably the method of settling dominance. Such aggressiveness has been reviewed by Collias (1944). The bird which regularly loses the fights is inferior to the winner and ultimately defers to his superior. The importance of an understanding of the fighting lies in the situation produced occasionally when a female does not, for the moment, correctly identify the sex of the male. In this case, she raises her neck hackle, thus inciting the male to start a fight instead of seeking to copulate. Such errors of identification on the part of the female are rare.

BEHAVIOR OF FEMALE TOWARD FEMALE

When two unacquainted females are placed in a pen, they begin to fight in the same manner as do the males. The neck hackle is elevated, and the birds strike each other with beaks and shanks. However, the vigor and persistence of the fighting is usually markedly less intense than is that of the males. Occasionally, two unacquainted females merely threaten each other and do not fight at all, though this is a rare occurrence. As in the case of the male, the result of this fighting is the settling of dominance.

SEX DISCRIMINATION

The method by which an individual recognizes the sex of a newcomer has interested many investigators. Most birds identify the sex of an individual by its behavior. Whitman (1919) stated that "the behavior is the only guide they [pigeons] have in selecting a mate of the right sex." Allen (1934) found that the slightly dimorphic Ruffed Grouse would copulate with an individual in the copulatory position no matter what the plumage was. Noble and Vogt (1935) found that the Golden Pheasant would mount a stuffed female of two other species somewhat resembling the female Golden Pheasant but would not mount a male Golden Pheasant. They concluded

that the greater the divergence from the female Golden type, the less the persistence of courtship. But, on the other hand, in sexually dimorphic birds Noble and Vogt (1935) found that at least two species, the Maryland Yellowthroat (*Geothlypis trichas*) and the Eastern Redwinged Blackbird (*Agelaius phoenecius*), distinguish sex by the appearance of the plumage. There is some evidence that this is a learned response because young individuals are less expert in differentiating between a male and a female. Noble (1936) carried the work further and found that in the Flicker, the sex is distinguished by the presence (in the male) or the absence (in the female) of the black feathers near the mouth (the "moustache"). Birds, however, by means of minute particulars soon become acquainted with other individuals as individuals and do not require a specific behavior at each meeting for recognition, as is illustrated in the Snow Bunting (Tinbergen, 1939*b*).

During this investigation numerous observations were made on the method of sex discrimination, although no experiments were conducted with this problem in mind. Even though the Brown Leghorn is highly dimorphic in plumage, the discrimination of sex occurs on the basis of behavior. If a strange bird raises its neck hackle, even though it is a female, it is considered a male by the male and is attacked. If the bird squats, it is considered a female; but if it runs away, it is considered an inferior in the peck-order and is let alone. However, very active roosters will occasionally pursue and attempt to copulate with a nonreceptive female or even another rooster.

In order to facilitate discrimination, the rooster has a method of enticing the newcomer to indicate, by behavior, its sex. When confronted by a bird which neither squats nor raises the hackle, the rooster waltzes or dances around the bird with the inner wing lowered and scraping the shank and the floor. If it then squats, he copulates; if it raises the hackle, he fights. Commonly, the rooster will waltz many times before a nonreceptive female in his attempt to get her to display her sex. This waltzing has been interpreted as an attempt to get the female to squat. This interpretation does not seem to be tenable, since the rooster can recognize the bird as a female only if she squats. He determines sex by whether or not the bird squats. A bird appears to be neuter or sexless, until by behavior it indicates its sex.

The normal rooster seldom takes time to waltz before the dummy. His impulse to copulate is so great that he copulates at once, even though the dummy is not a perfect replica of a squatting female and —what is even more important—does not move. It is very likely that roosters learn by experience as they get older to distinguish sex on the basis of plumage. This would seem to be indicated by the fact that two strange roosters commonly began to fight without the preliminary waltzing and that normal roosters frequently copulated with females without the usual preliminaries. The relation of learning to the method of sex discrimination introduces a series of profound problems which we shall not discuss here.

DISPLACEMENT REACTIONS

Animals which are prevented from carrying out one behavior pattern frequently perform a substitute pattern. Tinbergen (1939*a*) points out that substitute activity or displacement reactions (Tinbergen and van Iersel, 1947) serve as an outlet for inhibited activity. Common examples of substitute activity in birds are preening and building scrapes or play nests (Tinbergen, 1940) and picking up nesting material during threat

display (Goethe, 1937). When a rooster is prevented from copulating, he frequently goes through a performance of calling and pecking at the floor which has been termed "tidbitting." In teleological terms he appears to be trying to entice the female to come to him for the imaginary piece of food. In normal roosters this performance is fairly common. They commonly tidbit before a nonreceptive female or after several copulations. Another behavior pattern displayed by roosters as a displacement reaction is scratching for food. When a bird is feeding on the floor, he scratches away the shavings or other material adjacent to him in a characteristic manner, using about three strokes with his feet and holding his head upright. Following the scratching, he lowers his head to look for a morsel of food. The intersexual males commonly mounted the dummy, but, instead of continuing the copulatory behavior, scratched violently in a manner behavioristically identical with the scratching for food. However, these individuals do not complete the pattern by lowering the head to pick up food.

DESCRIPTION OF SEXUAL BEHAVIOR OF INTERSEXUAL BIRDS

The sexual reactions of the intersexual males showed gradations from essentially perfect masculine to definitely neutral or inactive behavior patterns. Tables 1–4 give a summary of all pertinent data. Reference should be made to the description of normal behavior for contrast.

BEHAVIOR PATTERNS

The present series of experiments provided an opportunity to divide the behavior into several components. A progressively more complicated and cumulative series of behavior patterns coincided with the degree of masculinity of the plumage and of the behavior as a whole. Several stages in the development of the waltzing behavior preliminary to copulation may be distinguished: (1) The lowered wing. The bird lowers the wing to the ground and bends the head; the action then ends, and the bird may walk away. (2) Waltz and stop. The bird lowers one wing and kicks backward and sideward with one foot, then abruptly rears up and stops. (3) Waltz and circle. The bird lowers the wing, kicks in the typical manner, and then circles around the female. (4) Waltz, circle, and grab. The bird waltzes in a perfect manner and grabs the female by the neck hackles or comb. This last pattern is sometimes followed by stage 5, the complete copulatory behavior. It must be remembered that the waltz is a behavior pattern associated with sex discrimination and that the "circle and grab" is a preliminary to copulation. There is, therefore, a sequence of two distinct behavioristic entities.

The completeness of the behavior pattern of an individual bird may vary on different occasions. For example, a male may usually show only stage 3 but sometimes go as far as stage 4. In general, however, the birds of Class IV seldom went beyond stage 1 (see Tables 1–4). The birds of Class III usually proceeded no further than stage 3. Birds of Class II commonly performed stage 4. Birds of Class I copulated occasionally (stage 5).

Another behavior pattern requires careful analysis. The waltz and peck consists of a complete waltz followed by a vicious peck on the head or neck of the female. The bird appears to be performing the waltz and grab, but at the last moment pecks instead of grabs. The pecking has the characteristics of the fighting between females. The interpretation of this behavior as a displacement reaction is discussed below.

The intersexual birds frequently

mounted the live female or dummy but, instead of continuing the copulatory behavior, scratched violently in a manner behavioristically identical with the searching for food. One female lost an eye owing to such scratching. However, the birds did not complete the pattern by lowering the head to pick up food. This behavior follows a waltz and circle and appears to be a displacement reaction.

The intersexual birds performed tidbitting, which is considered a displacement reaction, very much more often than did normal males. This would indicate that these birds were in a frustrated state nearly every time a female was placed before them.

REACTIONS OF INTERSEXUAL MALES TO NORMAL FEMALES

The intersexual males were tested with females, following the procedures outlined above (p. 16). In describing their behavior, typical cases will be cited from each of the four plumage classes.

Class I.—These birds, whose plumage resembles that of a normal male, showed the most masculine behavior. Many individuals crowed regularly, were active and aggressive, and waltzed frequently. The following selections from forty such observations in the protocol indicate the type of behavior observed:

Bird No. 1603T was removed from the pen on April 29 at 4:00 P.M., and returned on April 30 at 10:35 A.M. with female 32, a good layer. He behaved as follows: Waltzed, stood over female, trampled, scratched, and kicked her, although she squatted well. Waltzed vigorously, tidbitted, walked away. He returned, tidbitted vigorously, waltzed vigorously, but female ran away this time.

A similar experiment using bird No. 1550W with female No. 288 at 9:30 A.M. on April 30 gave the following result: He approached slowly and waltzed many times. The female ran. He followed and waltzed, then fought when she raised her head.

Bird No. 1502M was isolated from his female penmates on June 4 and returned to them on June 6. He behaved as follows: He ran to "Female RU who squatted at once as he approached her. He did not waltz but mounted and copulated at once. He waltzed again and the female squatted. He stopped."

The following conclusion can be stated from this series of experiments: The males of Class I will copulate in some cases with a known female, especially if she squats when he seeks copulation. Isolation may be necessary to increase his tendency to copulate. When exposed to a strange female the Class I male performs the usual actions preliminary to copulation.

Class II.—These birds, as would be expected from the fact that the plumage is somewhat feminized, seldom copulated under experimental conditions and showed definitely less masculine sexual activity. None was known to produce fertile matings, as determined by tests with virgin females. The following examples are typical:

On May 23, No. 1544U was placed with a normal laying female, No. 342. He waltzed and circled twice, and the female squatted well. He tidbitted vigorously, without any further action.

On May 16, No. 1515W was put in a pen with a normal laying female. She squatted, and he waltzed but did not get hold of her comb or neck hackle when he grabbed. He waltzed poorly many times. Then the female ran.

On June 4, No. 1653P was returned to his own pen after an absence of two days. He waltzed and copulated with a known female at once. He then waltzed and circled many times and pecked her until she fled to the roost.

Class III.—These birds, although definitely female in general appearance, have some masculine plumage characters. The behavior is very abortive and confused as indicated by the following observations:

On April 25, bird No. 1570R was placed with a normal laying hen. He waltzed poorly several times, kicked but did not lower the wing in the usual manner. Stepped on female; stood over female. Crowed imperfectly twice, waltzed

TABLE 1*

BEHAVIOR OF CLASS I INTERSEXUAL MALES

BIRD No.	PEN No.	RANK†	BEHAVIOR TOWARD		
			Female‡	Dummy§	Male‖
1509P.....	10¶	1–3	Copulated when female squatted; otherwise waltzed and pecked	Waltzed and pecked	Won against a rooster in own pen but lost in strange pen
1603T.....	6	1–4	Waltzed vigorously, trampled female when she squatted	Waltzed and pecked	Lost to rooster
1550W.....	9	1–5	Waltzed perfectly, copulated with squatting female	Waltzed and pecked	Started fight
1552B.....	9	5–1	Waddled (had enlarged oviduct)	No action	No action
1522T.....	10¶	2–2	Copulated; waltzed and pecked	(Became sick)	.....
1502M.....	11¶	1–5	Copulated with own squatting female	Pecked and fought	Won once; lost once
1684B.....	14	1–3	Grabbed and trampled	Pecked and stood; waltzed and pecked	Ran
1724P.....	16¶	1–5	Waltzed well; crowed often	Gave alarm cackle	Won in own pen
1728U.....	16¶	2–4	Waltzed well; tidbitted violently	Waltzed and pecked	Won in own pen

* Class I birds were the most masculine of the intersexual males and were essentially indistinguishable from normal males on the basis of external appearance.

† The rank in the social order of each pen is given here. The first number in each instance is rank from the top, the second rank from the bottom.

‡ The actions when exposed to a normal female.

§ The actions when exposed to a stuffed female mounted in the squatting copulatory position.

‖ The actions when exposed to a normal male or to an intersexual male.

¶ Females in these pens laid fertile eggs.

TABLE 2*

BEHAVIOR OF CLASS II INTERSEXUAL MALES

BIRD No.	PEN No.	RANK†	BEHAVIOR TOWARD		
			Female‡	Dummy§	Male‖
1503W.....	10¶	3–1	Waltzed and stopped; waltzed and pecked	Waltzed and circled	Won in own pen; won against Class II, III, and IV birds in new pen
1589PW.....	7	3–3	Walked around; lowered wing	Walked around	Lost to rooster
1544U.....	9	2–4	Trampled female when she squatted	Waltzed and circled	Ran
1515W.....	11¶	2–4	Trampled; waltzed and pecked	Waltzed and pecked	Won
1529U.....	11¶	5–1	Pecked viciously and trampled her	Pecked viciously	Behaved like a nonreceptive female; ran
1653P.....	13¶	1–3	Copulated with own female; waltzed and pecked	Pecked, circled	Won in own pen

* Class II intersexual males display considerable feminization of plumage, though this is still predominately male. The other sex characters are male.

†, ‡, §, ‖ Procedures were those indicated in Table 1.

¶ Females in these pens laid fertile eggs.

adjacent to female, but she fought; waltzed again, but she ran. Crowed imperfectly twice. Tidbitted poorly.

On April 29, No. 1660M was placed with a normal laying female. He waltzed and circled several times. Pecked female. She squatted well. He stood still.

Class IV.—These birds, whose plumage is practically identical with that of normal females, behaved in a neutral or only partially masculine manner under the experimental conditions. Apparently the birds were able to perform only certain preliminary phases of the masculine behavior pattern.

When placed with a normal laying female on May 7, bird No. 1687P performed as follows: He approached her with lowered wings. Pecked and trampled her. Stood over her.

Bird No. 1604 when placed with a normal laying female, on April 22, showed little activity. He looked at the female when she came near and trampled her. Waltzed very imperfectly. Stood still most of the time.

Bird No. 1623U when placed with a normal laying hen on April 30, performed as follows: Pecked female hard twice, waltzed poorly. Tidbitted poorly. Tidbitted well but briefly. Waltzed but, when female squatted, pecked her comb hard.

REACTIONS OF INTERSEXUAL MALES TO THE DUMMY

Since the stuffed hen, mounted in the squatting position of invitation to copulation, provided an object stimulus which was unvarying, the response of the males to such a stimulus was a more reliable measure of sexual activity than was their response to a live bird. In general, the experiments agreed well with those performed with normal females, but for each class the behavior patterns were on a lower level of activity.

Class I.—These birds, the most masculine in plumage, generally waltzed and pecked the dummy. Occasionally, they walked around it and stood over it. No individual copulated with the dummy. The following example was typical:

On June 25, at 10:40 A.M., No. 1684B was introduced to the dummy. He waltzed and pecked three times. He then stopped and walked around it. Then he waltzed and pecked again before walking away.

Class II.—These somewhat feminized birds generally waltzed and circled the dummy. Sometimes an individual pecked the head in a vicious manner. The following is a typical case:

Bird No. 1544U, on June 21, ran to the dummy and then waltzed and circled. He peered at it and then tidbitted. On June 30 he waltzed and circled three times and then waltzed and stopped. He walked away.

These birds on the whole showed definitely less masculine behavior than did the individuals of Class I.

Class III.—When exposed to the stuffed dummy, birds of this class generally walked around it or looked at it and occasionally pecked its head. The following are representative observations:

Bird No. 1612Y, when introduced to the dummy on June 24, peered at it for a moment and then walked around it. Soon he began to "tidbit" and eventually pecked its head gently. As another example, on June 21, bird No. 1519P waltzed poorly in front of the dummy and then pecked its head gently. He stood still, walked around, pecked it again and then tidbitted.

Class IV.—The birds of this class, which showed the greatest degree of feminization, showed little interest in the dummy. They usually perceived it, nevertheless, and walked over to it. In a few instances an individual waltzed poorly before the dummy. A typical example may be cited:

On June 24, bird No. 1624V walked around the dummy and then stood still a moment before beginning to tidbit. The next day, however, it waltzed poorly, stopped, and then walked away.

It should be emphasized that the birds of Class IV did react to the dummy but without any definite sexual reactions.

TABLE 3*

BEHAVIOR OF CLASS III INTERSEXUAL MALES

BIRD No.	PEN No.	RANK†	BEHAVIOR TOWARD			REMARKS
			Female‡	Dummy§	Male‖	
1612Y.....	6	3–2	Waltzed poorly; tidbitted violently	Walked around; tidbitted; circled	Ran	
1614U.....	6	2–3	Stood over female	Circled around	Attacked but ran	
1610R.....	7	4–2	Waltzed	Walked around	Lost	
1540BW...	9	4–2	Waltzed; when female squatted, stepped on her and pecked her	Pecked; raised hackle and fought	Squatted but soon got up	Pecked hens in pen in the female manner
1519P.....	11¶	4–2	Waltzed and pecked even when female squatted; ignored females	Waltzed poorly; pecked head	Won a fight in own pen	
1570R.....	13¶	2–2	Waltzed poorly; waltzed, circled, and pecked	Walked around; pecked head gently	Ran	
1660M....	13¶	3–1	Waltzed and stopped or pecked	Walked around	Ran	
1705PW...	16¶	5–1	Waltzed poorly; circled,	Walked around; waltzed and circled	Ran; little action	
1717PU...	16¶	3–3	Waltzed; pecked, waltzed, and stopped	Lowered wing; waltzed, pecked	Ran	

* In Class III intersexual males the plumage was predominately feminine. Head furnishings and spurs were male.

†, ‡, §, ‖ The procedures were those denoted in Table 1.

¶ Females in these pens laid fertile eggs.

TABLE 4*

BEHAVIOR OF CLASS IV INTERSEXUAL MALES

BIRD No.	PEN No.	RANK†	BEHAVIOR TOWARD			REMARKS
			Female‡	Dummy§	Male‖	
1604BW...	6	4–1	Walked around	No reaction	Started to fight but ran	
1623U.....	7	5–1	Waltzed poorly or stood still; when female squatted he pecked her	Walked around; peered at dummy	Ran	
1624V.....	7	2–4	Walked around	Walked around; waltzed and stopped	Won in own pen	Fighting and sexual activity not of same degree
1547PU...	9	3–3	Trampled; pecked when she squatted	Stood, pecked	Won in own pen	Pecking in female manner
1516B.....	11*	3–3	Stepped and trampled; no waltzing	Pecked, stood	Ran	
1687P.....	14	3–1	Waltzed and pecked; trampled; ignored female	Waltzed poorly; pecked	Ran after receiving 5 spurrings	
1706.....	14	2–2	Trampled and pecked; ignored female	Waltzed and pecked	Soon lost fights	

* Class IV birds were the most feminine of the intersexual males. The plumage of these individuals was almost completely, and in some cases completely, feminized. Head furnishings were frequently more like those of the female than like those of the male. Spurs were well developed.

†, ‡, §, ‖ Procedures were those designated in Table 1.

¶ Females in this pen laid fertile eggs.

Completely gonadless birds of both sexes did not appear to see the dummy but ignored it completely. In other words, the intersexual males of this category apparently perceived the dummy, whereas, in contrast, capons and bilaterally ovariectomized poulards showed no reaction whatsoever to it.

One result of particular interest is that the intersexual males reacted more strongly to a moving or especially a squatting bird than to a stationary one. For example, the only case of partial copulation with the dummy occurred on June 21 by male No. 1550W, which belonged in Class I. To quote from the protocol:

> When I allowed all the birds to enter the pen, several of them pecked the dummy's head very hard, causing it to move, whereupon 1550W immediately mounted in reverse position. I suspect that the motion was sufficient to incite this intersexual male to mount.

It would therefore seem that the actual movement of the female in assuming the squatting position is a more potent aphrodisiac than is an object which is already in that position.

REACTIONS OF INTERSEXUAL MALES TO NORMAL MALES

The reaction of an intersexual bird when confronted with a normal male again parallels the character of its plumage. Birds of Class I regularly raised the neck hackle and attacked a new male. However, they usually lost the ensuing battle. Birds of Class II sometimes began a fight but soon gave up. Birds of Classes III and IV seldom fought but merely ran away from an intruding male. As was true in the case of peck-order, a bird in its own home pen, with its own mates removed, had a better chance of winning than when placed in a new pen. This fighting, of course, cannot be considered sexual behavior as such but is merely the method of determining the social rank and is the alternative to sexual behavior.

ONTOGENY OF BEHAVIOR

In order to study the development of behavior in young birds a series of pens, each containing about 10 immature intersexual males, was organized. These were divided into four groups, containing birds approximately 3, 4, 5, and 6 months old. The birds in each group were tested in the same manner as has been described above for adult birds. A normal male (control) of the same series was present in each pen.

The intersexual males, as compared with the normal males, were greatly retarded in the development of sexual behavior. Only a few individuals at 5 months of age showed any sexual activity. The normal male, in contrast, crowed and copulated regularly at this age. Intersexual birds at the age of 6 months never copulated under the experimental conditions; nevertheless, they showed definite sexual activity, although of an abortive type. Their behavior consisted of the patterns of waltz and grab or merely a waltz and peck. Frequently, they trampled and scratched the female or dummy violently. These behavior patterns appeared identical in form to those of adult intersexual individuals. It would appear that the gonads of these immature intersexual males do not produce adequate hormone to induce the sexual behavior patterns of a normal individual of the same age.

FERTILITY

To test their fertility, 18 mature intersexual males, 22–24 months old, were individually left in a pen overnight with a normal virgin female. Of 106 eggs obtained, only 3 (all from same male) were fertile. In the case of normal roosters of this flock, 75–80 per cent of the eggs were fertile.

REACTIONS OF EXPERIMENTAL FEMALES TO NORMAL MALES

Females derived from estrogen-injected eggs of this same series developed normal plumage. In this paper they are referred to as "experimental females" to distinguish them from normal birds derived from untreated eggs. The experimental females behaved in an essentially normal manner when placed with a normal male. Generally, those which were laying regularly squatted for copulation. Those which seldom laid, or did not lay at all, usually because of oviducal abnormalities (Domm, 1940*b*), avoided the male and fled when he attempted copulation.

REACTIONS OF EXPERIMENTAL FEMALES TO INTERSEXUAL MALES

Owing to the fact that the intersexual males generally approached the female in a confused and imperfect manner, seldom waltzing correctly and frequently pecking and injuring the female, the females learned to keep away from the male. However, if the male approached in a sexually active and perfect manner, the receptive females squatted for copulation in the normal way. That copulation occurred fairly regularly among some of these individuals is demonstrated by the fact that 15 females out of 28 distributed among four regular pens laid fertile eggs. Two of the pens contained 5 and two 6 intersexual males, some of which belonged to Class I. The fertile eggs were laid between February 1 and July 31, 1941.

DISCUSSION

The variability of animal actions is a constant source of difficulty in any experimentation that attempts to analyze behavior patterns. The same bird may, on various occasions, behave quite differently. Apparently, the number of factors influencing the actions is great, and under experimental conditions it is not yet possible to control all these factors. Slight noises, cloudy weather, movements by the experimenter, calls of other birds, and numerous other external variables may be responsible for moment-to-moment variability. Then, in addition, there are numerous internal factors, such as recency of eating or slight changes in physiological state, which may alter the actions from day to day. Still a third group of factors may be described as "psychological." Learning and conditioning, because they are dynamic processes, are constantly occurring and thus may result in changes in psychological state on subsequent days. These three general groups of factors are the source of variability which is normal in the behavior of animals. For this reason it is not surprising that under apparently identical conditions the day-to-day record of one bird shows a considerable range of variation. Nevertheless, in spite of this variation, it is possible for the trained observer to detect the common action of the individuals and make generalizations which have as much validity as those derived from quantitative data.

It is now well established that the effectiveness of hormones is dependent on levels of concentration, as well as on the threshold of the reaction system. This principle is demonstrated very nicely by the well-known studies of F. R. Lillie and his associates on the plumage of the Brown Leghorn. They observed that the various feather areas, as well as the parts of individual feathers, have different thresholds of hormone reaction and that these thresholds are determined by growth rates, the more rapidly growing feather areas, or parts of individual feathers, having higher thresholds than the slower-growing ones.

Apparently, behavior patterns also

have specific thresholds and come into function at varying levels of hormone concentration. The evidence presented in this paper suggests that the birds in each of the classes considered had different amounts of sex hormones and were thus limited in the behavior patterns which could be manifested. A likely hypothesis is that the birds in Class IV possessed only enough sex hormones to perform certain patterns, whereas the birds in Class I possessed enough to perform nearly all behavior patterns.

The problem of thresholds has been approached in this case by alteration of the relative amount of ovarian and testicular tissue in the individual. The problem can also be attacked by the removal of part or all of the testis as was done by Carpenter (1933) in pigeons. He found that a reduction of as much as 80 or 85 per cent in the normal amount of testicular tissue did not preclude normal sexual behavior (copulation). Further reduction reduced the frequency of copulation. In Carpenter's opinion, "the hierarchy from the higher to the lower thresholds is in the following order: copulation, charging, billing and preening." The latter three are secondary sexual behavior patterns of the pigeon. In the cock, Benoit (1929) found that the behavior patterns had higher thresholds than did anatomical structures and that the order from higher to lower thresholds was (1) fighting, (2) crowing, (3) sexual instinct. Roberts (1940) found that the sexual organs of penguins were completely matured before the behavior patterns reached perfection, thus suggesting that the threshold for copulatory behavior is higher than that for accessory reproductive organs.

A low concentration of hormone apparently results in displacement reactions, which occur when another pattern is impossible. It would seem as though nervous energy is built up and, being prevented from being expended in one direction, must be released in another. One of the characteristics of such performances is that the pattern occurs with greater force than it does under normal conditions. In these experiments the tidbit was performed with great activity and frequent calls. In addition, the scratching—another displacement reaction—was most violent, far more so than in normal feeding.

One additional behavior pattern may also be a displacement reaction. Frequently, as described above, the intersexual male pecked the head of the dummy or normal female after waltzing. This pattern is the action of fighting as performed by a female. At present it is impossible to decide whether this pecking is a displacement reaction or is a fighting action brought about by the presence of androgenic hormone in the body.

The actions of an individual are determined by two general types of conditions. The first is the physiological and psychological state of the individual, and the second is the character of the stimulus. Unfortunately for the investigator, the two types are seldom stable. Each is dependent upon a number of factors, all of which change from moment to moment. However, it is possible to single out certain observations and group them under several factors. The sexual behavior seems to be, at least in part, dependent upon the location factor. Birds in new locations or flocks require some time before they will perform in the normal manner. A familiarity with the pen seems to predispose the bird to greater sexual activity. Another factor which seems to have an influence on the sexual activity is the position in the peck-order. In this series of experiments no

observations were made on the relation of peck-order to sexual activity, but it is suggested that the low activity of the highly feminized birds may have been in part due to the low rank in the peck-order. Birds low in the social rank are timid and habitually run from their superiors. Possibly they are, in reality, psychologically castrated. A third factor of significance is the previous history of the bird. Individuals low in activity may require some time before they are able to assert their rightful position. The conditioning which has occurred in the past history influences the behavior greatly.

The observations recorded in this paper present an interesting comparison with the seasonally maturing sexual behavior of wild birds. It is a matter of common knowledge that many birds, in the beginning of the breeding season, incompletely perform certain patterns. For example, a bird may pick up a stick and carry it about and then drop it; or it may start one nest one day and another nest the next; or it may select a territory and subsequently abandon it. This abortive behavior is the rule during the first part of the nesting season. The behavior observed in the intersexual roosters in our experiments resembles that of the wild birds in principle, the essential difference being that in the intersexual fowl, the gonad does not ultimately produce the requisite amount of hormone, and hence the bird is unable to perform the complete normal behavior pattern. Noble and Wurm (1943) believe that incomplete copulatory behavior in the laughing gull may be the result of incomplete dominance between male and female.

This series of experiments presents further observations on behaviors which have been called "releasers" (Lorenz, 1935). A "releaser" may be defined as a display by one individual which elicits or releases a response in another individual. When one rooster raises the neck hackle, the other male automatically raises his, and a fight ensues. The waltz is a releaser, which may result in either of two reactions, namely, squatting by the female, followed by copulation, or raising the hackle by the male, followed by fighting. These two behavior patterns seem to be releasers in the original usage of the term.

SUMMARY

1. The behavior of intersexual domestic fowl, produced by injection of estrogenic hormones into the egg, was examined from the psychoanalytical viewpoint by testing the birds with a normal rooster, a normal female, or a stuffed female dummy, mounted in a squatting pose of invitation to copulation. The birds showed varying degrees of masculinity of the plumage and were accordingly divided into four arbitrary classes.

2. As is true of groups of normal domestic fowl, the intersexual birds of each pen had a peck-order or social hierarchy. The rank of the males roughly coincided with the degree of masculinity of the plumage.

3. In order to understand the abnormal behavior of the intersexual birds, the normal behavior of normal fowl was examined. To entice a bird to indicate its sex, the rooster waltzes (lowers one wing and kicks). If the bird is a receptive female, it squats for copulation. If, on the other hand, it is a male, it raises the neck hackle, thereby beginning a fight.

4. The sexual behavior of the intersexual males showed varying degrees of perfection, roughly according to the masculinity of the plumage. Birds of Class I copulated occasionally and waltzed and crowed regularly. Birds of Class II seldom copulated but performed the pre-

liminary actions. Individuals of Class III acted in an abortive manner but waltzed. Birds of Class IV showed only the most preliminary behavior patterns.

5. Intersexual males showed a retardation in the development of sexual behavior in comparison with normal males.

6. Only a few of the most masculinized of the intersexual birds belonging to Class I were fertile.

7. Experimental females derived from estrogen-injected eggs behaved essentially in the normal feminine manner.

8. In the discussion it is suggested that the behavior patterns depend upon the level or threshold of hormone concentration. Furthermore, it is notable that the abortive behavior of the intersexual males greatly resembles the seasonally maturing sexual behavior of wild birds.

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DOMINANCE AND THE FRUSTRATION-AGGRESSION HYPOTHESIS

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ONE of the chief advances of comparative sociology and social psychology in recent years was the discovery that other animals besides the insects and man were possessed of complex and intricate social organizations. This discovery began with the work of Schjelderup-Ebbe (1922, 1935) on dominance in chickens. This, which is only one of the many types of social organization now known to exist in vertebrates, is essentially a type of social organization based on fighting behavior. Through the work of Allee and his associates it has since been found that in almost every case in which social fighting is present in vertebrate animals this behavior is organized into a similar system (Collias, 1944).

This system is a relatively simple one, based on learning. When two animals fight for the first time, one usually wins and the other loses; and, if this situation is repeated several times, one gets into the habit of winning and becomes dominant, while the other forms the habit of losing and becomes subordinate. The end-result is that the dominant animal threatens, and the subordinate one retreats, greatly reducing the amount of actual fighting.

More recently it has been discovered that flocks of chickens which are well organized into a system of dominance get along much better than do similar flocks in which there is no such organization, presumably because the dominance organization has the effect of reducing the amount of fighting (Guhl and Allee, 1944).

During this same period Dollard and other workers at the Yale Institute of Human Relations (1939) have strongly advanced the hypothesis that the chief cause of aggression in our society is frustration. This idea apparently originated in the psychoanalytical studies of Freud, and there appears to be a great deal of animal and human data which supports it.

In order to examine this hypothesis critically it is necessary to consider the use of the terms "aggression" and "frustration." "Aggression" is defined by Dollard and his associates as "an act whose goal-response is injury to an organism (or an organism-surrogate)." "Goal-response" is, in turn, defined as "that reaction which reduces the strength of instigation to a degree at which it no longer has much of a tendency to produce the predicted behavior sequence." It is obvious from the context that they are not talking about defensive fighting, which is often caused by injury rather than by frustration, although its goal-response may also be injury to an organism.

"Frustration" is defined by the same authors as interference with the occurrence of an instigated goal-response at its proper time in the behavior sequence. In dealing with animals it is perhaps preferable to define "frustration" in terms of adjustment, so that frustration becomes interference or prevention of adjustment to any change, whether the change is internal or external. However, these definitions do not conflict.

The frustration-aggression hypothesis was later reviewed by Miller (1941), who stated that frustration does not always lead to aggression and suggested investigation of modifying factors which affect aggression. Since fighting is apparently almost universally organized into dominance relationships (Collias, 1944), it should be of interest to consider the effect of dominance upon the frustration-aggression hypothesis.

The reverse experiment—the effect of frustration upon dominance—has been tested by Seward (1945), who presented a food-frustration situation to rats already organized into a dominance system not previously connected with food. He found little effect on dominance except when the animals were eating from a small food hole, which prevented the simultaneous sight of food and the competing rat. However, Seward did find that hunger and competition had the effect of lowering the total amount of fighting behavior in this situation, and he was led to question the statement that aggression is always preceded by frustration. This point will be dealt with later in the discussion.

In an earlier study of the social organization in a "naturally" organized flock of sheep (Scott, 1945), it was observed that very little fighting took place. The animals were not fed in the usual fashion but were allowed to pick up their own food, and this reduced food frustration to a minimum. The fighting that did occur was chiefly observed during the breeding season, when many males attempted to mate with a single female in heat—an obviously frustrating situation. In general, these observations supported the hypothesis that frustration was the cause of aggression.

More recently the author had an opportunity to study a small flock of goats. This flock was not formed as naturally as the sheep, and the goats were being fed by the usual method of dumping a quantity of feed into a small area, with obvious frustration. It was observed that a good deal of fighting was taking place and that there were some indications of a regular dominance organization.

This was apparently a good chance to study a dominance organization; and, as this study went on, it was observed that the amount of fighting was very markedly affected by the recency of the last meal, which offered a direct opportunity to test the frustration-aggression hypothesis.

MATERIALS AND METHODS

The goats studied consisted of fourteen animals of mixed ancestry. Two of

these, a male and female, had been raised on the place as pets, and later two other mature females from different places were brought in. All the other animals were descendants of these four.

The flock fell into three age groups (see Table 1): the three old females and old male mentioned above; a group of two males and three females, which were less than a year old; and a group of four kids, which were less than six months old. There was one other male, which was slightly younger than the oldest group.

TABLE 1

MOTHER-OFFSPRING AND AGE-GROUP RELATIONSHIPS IN THE EXPERIMENTAL FLOCK

Approximate Date of Birth	Individuals Grouped by Maternal Lines*			
Unknown	A♀, A′♂	B♀	C♀	.....
Spring, 1944	.....	.....	C-1♂	.....
Spring, 1945	A-2♂	B-2♀, B-2′♀	C-2♀	D-2♂†
Fall, 1945	.....	B-3♀, B-3′♂‡	C-3♀, C-3′♂	.....

* In any individual, the figure after the hyphen indicates age group; figures and letter before hyphen indicate mother of animal; prime indicates a twin.

† Raised on bottle.

‡ Intersex.

Three of the animals had horns: one of the old females and one male and one female in the second group. The three age groups could be easily told apart by the relative size.

The animals were regularly fed each morning about nine o'clock, hay and grain being thrown out on the ground for them. Tests were always made during the afternoon. Most of the observations and tests were made during the months of December, January, and February, when cold weather and the shortage of natural foods apparently made the animals hungry.

In order to observe the system of dominance, the animals were taken a pair at a time and placed in a standard frustrating situation. The whole flock was driven into a pen from which they could see the other animals being fed. One pair was taken out at a time, and a small handful of grain or other prepared food was placed on a board about 6 inches square. This meant that the two animals would have to get their heads very close together in order to eat. In order to be sure that this situation was a frustrating one, the same observation was repeated after placing a handful of grain in a bucket into which only one animal could get his head.

Defining "frustration" in terms of adjustment, we can see that the situation is a complicated one. There is an internal change resulting in hunger, but no adjustment to this is possible until an external change occurs—the appearance of food. This adjustment is then interfered with or frustrated by a second animal, which gets in the way and obtains part of the food.

The pairs of animals were taken in random order, and only one test was given to each animal per day. In order to avoid the effects of training and in order to measure whatever dominance already existed, rather than to create or intensify it, the tests were not repeated until some months later.

After these results had been analyzed, the amount of aggression was measured in relation to greater and lesser degrees of frustration. The animals were shut up without food during their normal morn-

ing feeding time and allowed to go approximately 6 hours without food. They were then given the dominance test, and the number of aggressive acts (butting without previous butting by the other animal) were counted while they ate the small amount of food furnished. They were then fed heavily for a period of approximately 20 minutes (usually not to the point of satiation), and the dominance test was repeated.

In selecting animals for the latter test, each type of dominance was considered separately, and as far as possible every animal was included at least once in both dominant and subordinate roles.

DOMINANCE

As soon as the experiment was started it was obvious that the degree of dominance differed considerably between different pairs of animals. Some goats ate peacefully together, others fought vigorously for long periods, and still others parted after one or two encounters. The dominance reactions observed were then classified on the following basis:

Type

0. . . . No dominance; the two animals ate peacefully side by side.
1. . . . Unsettled dominance; both animals exhibited aggressive fighting.
2. . . . One animal was responsible for all aggressive fighting; the subordinate animal might exhibit a small amount of defensive fighting and stayed with the other animal until the food was finished.
3. . . . The dominant animal exhibited all the aggressive fighting, the subordinate animal eventually leaving the scene before the food was finished.
4. . . . The subordinate animal left the scene immediately after being threatened or being struck by the dominant animal.

Out of the 91 possible combinations of animals, there were observed 22 cases of peaceful behavior, 7 cases of unsettled dominance, 24 cases of type 2 dominance, 32 cases of type 3, and 6 cases of type 4. It will be observed that there was a considerable proportion of cases in which no fighting occurred.

DOMINANCE ORGANIZATION IN THE FLOCK

Dominance may now be considered in relation to the general organization of the flock. Facts are not available for a complete analysis of social organization in goats, although some progress has been reported in this direction in another paper (Stewart and Scott, 1947). In the present instance only the dominance organization of the flock will be considered (see also Scott, 1946).

As Carpenter has pointed out (1945), one of the best ways of analyzing social organization is to do so on the basis of relationships between individual animals.

A preliminary survey of goat behavior indicates that the important relationships are much the same as they are among sheep (see Table 1). There are three general types of relationships: (*a*) between older and younger animals, (*b*) between one female and a number of males or one male and a number of females, and (*c*) between animals in the same age group. There is one specific relationship—that between mother and offspring.

In such a small flock as this the only relationship which occurs in sufficient numbers that conclusions regarding it can be drawn is that between older and younger animals (see Table 2). Out of the 91 pairs, there were 69 cases in which older and younger animals were involved. Fifty of these showed dominance of types 2–4, and, of this number, the older animal was dominant in 47 cases. The conclusion may be reached that part of the relationship between younger and older animals is a strong tendency for the older animal to be dominant.

This definitely applies to dominance in feeding situations and also seems to apply to dominance in a sexual situation. Some months after the tests on feeding were made, the dominance between six mature males in the flock was observed at the time female A was in heat. In this case there were thirteen combinations of older and younger animals, and in ten of these the older animal was dominant. In one case the dominance was unsettled, and in two others the younger animal was dominant. It may be added that the exceptions to the rule all involved one younger animal with large horns, which seemed to give him considerable advantage in fighting.

Comparing the individual dominance relationships, it was found that nine out of fifteen were the same in the feeding and the sexual situations. Of the remainder, three were peaceful in the feeding situation. There was one case of previous unsettled dominance and only one case in which dominance was reversed. It seems likely that there is some correlation between dominance in these two situations (see Table 3).

TABLE 2

DOMINANCE STATUS BETWEEN OLDER AND YOUNGER ANIMALS

Animal	Older Animal Shows Dominance of Type:			Younger Animal Dominant
	0	1	2–4	
A ♀	1	0	9	0
A′ ♂	8	1	1	0
B ♀	0	2	7	1*
C ♀	4	0	5	1*
C-1 ♂	1	1	6	1*
A-2 ♂	0	0	4	0
B-2 ♀	1	0	3	0
B-2′ ♀	0	0	4	0
C-2 ♀	0	0	4	0
D-2 ♀	0	0	4	0
Totals	15	4	47	3

* A-2 ♂ with horns dominant.

There is, however, one curious difference. If the males are ranked for their dominance in the sexual situation according to the number of animals dominated, the oldest and largest male is found at the top and the other older males come close after him. On the other hand, in the feeding situation this male ranked only fourth among the males and tenth in the whole flock. The explanation appeared to be that he had formed a habit of standing his ground and eating rather than fighting over food, since he was not subordinate in any case.

TABLE 3

DOMINANCE SCORES AND RANK OF MALES ONLY, BY AGE GROUPS IN SEXUAL SITUATION AND FEEDING SITUATION

(Dominance Score = Number of Other Males Dominated)

Animal	Sexual Situation		Feeding Situation	
	Dominance Score	Rank	Dominance Score	Rank
A′ ♂	4	1	1.5	4
C-1 ♂	3	3.5	3	2
A-2 ♂*	3.5	2	4	1
D-2 ♂	3	3.5	2.5	3
C-3 ♂	1.5	5	1	5
B-3′ ♂†	0	6	0	6

* With horns. † Intersex.

Though the numbers concerned are small, it is probable that the male-female relationship is correlated with dominance. Aggressive relationships between mature males and females are about as common as within the whole flock, but the small numbers available indicate that there is a tendency for males to be dominant. In eleven cases of dominance belonging to types 2, 3, and 4, the males were dominant in nine cases, the two exceptions both concerning A, the old horned female.

FRUSTRATION, AGGRESSION AND DOMINANCE

With this picture of dominance organization in the flock it is possible to go ahead and consider the result of varying the amount of frustration. When animals are starved for a few hours, casual inspection shows two effects (see Tables 4 and 5). The formerly subordinate animal will take more punishment than before, and the formerly dominant animal shows increased vigor and prolonged fighting. Then, when the animals are fed, the result is a marked diminution in the amount of fighting and the number of cases in which dominance is apparent.

TABLE 4

EFFECT OF FEEDING ON AMOUNT OF AGGRESSIVE FIGHTING

Type	Before Feeding		After Feeding		Total Individuals
	Total	Ave.	Total	Ave.	
0	42	1.8	3	0.1	24
1	94	6.7	7	0.5	14
2	70	3.5	23	1.1	20
3	78	3.3	10	0.4	24
4	9	1.1	3	0.4	8
All types	293	3.3	46	0.5	90

As may be seen from Table 5 the most common effect of feeding on dominance is to eliminate it altogether, so that the two animals eat peacefully together. The next most common result is to increase the degree of dominance, presumably because the subordinate animal, having been fed, is now less resistant. There are no cases in which the degree of dominance was lowered by feeding, and the conclusion must be reached that there is a threshold with regard to feeding. On one side dominance exists, and on the other the two animals will eat peacefully together.

This effect may be checked (see Table 6) by comparing the previous dominance status with that seen when hunger is more extreme. The most important effect is on the previously peaceful cases. Only six out of twenty-four remained peaceful. Three previously subordinate animals became dominant, and only two previously dominant animals became subordinate; in the majority of cases their status remained the same. It is interesting to note that a large proportion of the previously peaceful animals showed a condition of unsettled dominance.

TABLE 5

DOMINANCE STATUS BEFORE AND AFTER FEEDING

Status before Feeding	Status after Feeding	No. Cases
Dominant	Same rank	12
	Lowered rank	0
	Increased rank	9
	Became peaceful	26
	Became subordinate	3
Subordinate	Remained subordinate and nonresistant	18
	Remained subordinate and resistant	1
	Became nonresistant	7
	Became resistant	1
	Became dominant	0
Peaceful	Remained peaceful	4

It is fairly obvious that dominance can be eliminated or brought into being by controlling the amount of food which is given. Presumably this, in turn, is caused by the effect of feeding on aggressive fighting. As can be seen in Table 4, there is no doubt that aggression is reduced by feeding. The total number of attacks after feeding was reduced by approximately 85 per cent.

There remains to be considered the effect of dominance (which ultimately involves training) in controlling and

modifying the amount of fighting that is presumably caused by frustration. This control appears to be very important. In the first place, the amount of fighting is inversely proportional to the degree of dominance. The greater the degree of dominance, the less fighting takes place. Eleven cases of type 1 dominance showed 112 attacks, while 6 cases of type 4 dominance showed only 7 attacks.

Second, the status of subordinate animals is scarcely changed at all by variations in the amount of frustration, even under conditions of extreme hunger.

Finally, there are pairs of animals which eat peacefully together under any conditions, and it follows that frustration tends to cause or at least increase aggression in situations in which animals are in the *habit* of being aggressive. As will be pointed out below, this conclusion has a great many broad implications.

DISCUSSION

In reading the earlier work on dominance, particularly the studies based on birds, it is easy to get an impression of a fixed and unchanging social order which may be properly called a "hierarchy." This conception was not borne out by early work on mammalian dominance (Uhrich, 1937), and the present study indicates that some mammals at least may show a variable system of dominance. The dominance relationship between any given pair of animals appears to be fairly stable over short periods, but even this may be occasionally modified as the animals develop and grow older. More important than this, dominance can be made to appear or disappear by modifying the situations under which the animals live. For example, if goats are given plenty of food which is scattered out to prevent crowding while eating, there should be no trouble, no fighting, and no dominance. This may be of practical importance to stock raisers in preventing the "hogging" of special food by certain animals.

It will be recalled that this experiment was set up so that each animal, as far as possible, would be represented in both dominant and subordinate roles. Yet there is no exception to the rule that it is only the dominant animals which become aggressive when frustrated. The same individual will be moved to aggression in a situation where it is dominant but will simply absorb more of a beating when subordinate.

TABLE 6

EFFECT OF INCREASED HUNGER ON DOMINANCE STATUS

Status on Previous Test	Status before Feeding	No. Cases
Dominant	Lowered rank	6
	Increased rank	8
	Became peaceful	2
	Became subordinate	2
	Remained same rank	21
Subordinate	Became dominant	2
	Became peaceful	2
	Remained subordinate	21
Peaceful	Became dominant	11
	Became subordinate	7
	Remained peaceful	6

In these experiments there is a very obvious relationship between the amount of frustration and the amount of aggressive behavior in the dominant animals. This seems to be contradictory to Seward's results in rats, but the apparent contradiction can be easily resolved.

The two experiments are really quite different. That described in this paper involves increasing the amount of food frustration in relation to a system of dominance already established on that basis, while Seward's experiment superimposed food frustration on a system of dominance established on another basis.

He found that the hungry rats tended to try to eat, which interfered with their habitual amount of fighting. Presumably, the use of a system of dominance developed around food frustration would give the same results in the rat as in the goat.

However, this does not answer the question raised by Seward as to what caused the rats to fight before food was denied them. The answer has great importance to the analysis of the causes of fighting but does not conflict with the conclusion of this paper. Because of the complexity of the evidence, its discussion must be deferred to a later paper.

The conclusion that frustration causes aggression in animals chiefly in situations in which they are in the habit of being aggressive does not decrease the importance of frustration as a cause of aggression, but it does increase the importance of training as a means of controlling aggression, a point which had perhaps best be amplified in connection with human affairs.

HUMAN APPLICATIONS

Any comparison between the psychological and social reactions of human beings and those of other animals must always be made cautiously, both with regard to genetic differences between the two species involved and particularly with regard to the fact that the development of speech in man makes for great differences in social organization. Within these limitations it is profitable to use animal experiments in two ways: (*a*) to experiment with similar factors in simple situations and (*b*) to regard the resulting conclusions as suggestive of new ideas which may apply to human situations. Both these things have been done in the present case.

Fighting between individuals in our modern civilized society is controlled to such an extent that dominance of the sort which is seen among nonverbal animals is rarely met with, except perhaps in small children who are allowed some freedom of fighting and whose aggression often escapes the bounds of training, and also between parents and offspring where physical punishment is permitted. It has often been alleged that hunger is a cause of fighting; and, if the same conclusions may be drawn regarding young children as those drawn from the facts of this experiment, it may be concluded that hunger and frustration should cause aggression in a child in a relationship in which he is dominant and hence in the habit of fighting. In order to confirm this assumption, it would be necessary to observe children in a common food-frustration situation, such as waiting for mealtimes. Does the child ever attack parents or older brothers and sisters, or is aggression always directed against small children, pet animals, and inanimate objects, over which he presumably dominates?

This again suggests an explanation for the commonly observed phenomenon of substitution in aggressive acts—the man who accepts aggression from an employer but takes it out on his dog, and the reprimanded child who attacks his small brother. In each case aggression seems to come out only in situations in which the aggressor is dominant. It would be interesting to test out this hypothesis using various animal societies.

Since training seems to be such an important factor in affecting the amount of fighting in animals, one wonders what its importance might be in human society. Would frustration be an important cause of aggression in a society like that of the Hopi Indians, where little aggression is permitted by social usage? Bateson (1941) has already raised this question in connection with Balinese culture. Again, what is the effect in our society of rules

regarding fighting which justify it in defense of person or property, and are not these likely to increase the amount of fighting which is caused by frustration?

On the positive side this experimental study tends to give support to the common-sense notion that people who are well fed, and hence not frustrated in this respect, are happier and less likely to harm others, and it also lends support to the idea that feeding the hungry is a step in the promotion of peaceful living. It also indicates that feeding alone is not enough and that training may be extremely useful in the control of aggression.

SUMMARY

1. This experiment was designed to test dominance as a possible modifying factor of aggression caused by frustration.

2. A flock of goats containing several age groups was used. After the dominance organization was determined, the effect of delayed feeding and recent feeding upon the amount of fighting between the members of each pair was observed.

3. The degree of dominance seen in each pair was graded. More than one-fifth of the relationships were entirely peaceful under ordinary conditions. The degree of dominance appears to be inversely proportional to the amount of fighting.

4. The relationship between older and younger animals is definitely correlated with dominance, the older animals being dominant in 47 out of 50 cases.

5. The dominance relationships between males appear to be approximately, but not entirely, the same in cases of sexual frustration as in food frustration.

6. Delayed feeding increases the amount of aggressive fighting in dominant animals, and recent feeding decreases the amount of aggression in dominant animals.

7. Delayed feeding causes subordinate animals to take more punishment and thus lower the degree of dominance, but it almost never causes aggression in them.

8. The above conclusions also apply to animals which are subordinate in one relationship and dominant in another.

9. It may be concluded that dominance strongly modifies the effect of frustration in causing aggression. Hence it may be said that frustration causes aggression in situations in which animals are in the *habit* of being aggressive.

10. Some possible applications to human affairs are discussed.

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RELATION OF SALINITY TO THE ACTIVITY OF THE SPERMATOZOA OF *GILLICHTHYS*, A MARINE TELEOST[1]

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THE purpose of this experiment is to analyze the effects of salinity on the activity of the spermatozoa of the long-jawed goby or mudsucker, *Gillichthys mirabilis*. Since this teleost is a marine species, its spermatozoa are subjected to an entirely different environment than are those of the salmon and trout, which spawn in fresh water. The latter are the fishes that have been chiefly used in determining the relation of osmotic pressure to the period of activity of the spermatozoa.

The activity of trout spermatozoa in river water is very brief (Gray, 1920). Since NaCl and Ringer solutions, as well as diluted sea water, prolong the activity of trout spermatozoa, Huxley (1930) suggests that the trout, though somatically adapted to fresh water, is imperfectly adapted to reproduction in that medium. Ellis and Jones (1939) believe the effects to be osmotic rather than ionic because salmon spermatozoa live for about the same length of time in solutions of either sucrose or sea water which have the same osmotic pressure. These authors think that the spermatozoa are not so ill adapted to their environment as Huxley indicates. Fluid from salmon eggs greatly lengthens the life of the spermatozoa. The spermatozoa must endure only a short contact with fresh water before they enter the more advantageous medium of the egg fluid.

[1] Contributions from Scripps Institution of Oceanography, New Series, No. 356. I am indebted to Dr. Frederick Crescitelli for helpful suggestions and to Dr. Carl L. Hubbs for revising the manuscript.

MATERIALS AND METHODS

Filtered sea water was prepared in stock solutions ranging from 10 to 200 per cent concentration. The sea water was diluted with glass-distilled water. Concentrations above 100 per cent were obtained by evaporation. Artificial sea water was made according to the formula proposed by Brujewicz (Sverdrup, Johnson, and Fleming, 1946, p. 186). This was diluted or concentrated to solutions of osmotic pressure similar to the sea-water solutions. Corresponding osmotic solutions were also prepared with glucose in distilled water and with sucrose to which $CaCl_2$ had been added. The pH of all solutions was determined with a Coleman glass electrode.

The gobies were taken during their period of greatest spawning activity, March–May (Weisel, 1947). The testes were removed from freshly killed fish, and equal portions were placed in watch glasses containing the solutions of different osmotic pressure. Testes from the same fish were used in each series of dilutions, and two to four series were run for each different solute tested. A large drop of the solution was put on a clean cover slip, and the piece of testis was immediately teased apart in the drop. A hanging-drop preparation was then made—well sealed with vaseline. The preparations were examined under the microscope. A record was kept of the time at which all movement of the spermatozoa ceased. Other measurements for the termination of the experiments were

found to be less uniform and subject to observational fallacies.

This procedure was used because it is difficult to strip milt from this fish. The testes are very small. The diameter rarely exceeds 2.00 mm., even during the spawning season. Also, the contents of the relatively large seminal vesicle are mixed with the spermatozoa if the fish is stripped. Since the vesicular fluid contains salts and a large amount of proteoses (Young and Fox, 1937), it was considered that this fluid would alter the osmotic pressure of the solutions more than teasing a small portion of testis in the respective solutions.

EXPERIMENTAL RESULTS

I. ACTIVITY IN SEA WATER

To determine the lowest salinity at which the spermatozoa become active, sea-water solutions were diluted in grades of 1 per cent from 10 to 40 per cent. Activity first commenced at 17 per cent. Feeble movements were exhibited in dilutions of 17–24 per cent. In all higher concentrations practically all spermatozoa were activated. However, activation was effected less rapidly in 25 per cent sea water than in the higher concentrations. In 25 per cent solution, 2–5 minutes were required to produce motility over the entire field, but, once activated, the spermatozoa displayed the same rapid motion as in the solutions of greater pressure.

Stock solutions ranging from 18 to 200 per cent were tested with spermatozoa from four fish. The spermatozoa of each fish survived longest in 25 per cent sea water. Figure 1 indicates the shortening in survival time from this optimum as the concentrations become greater or less. The curve falls much more rapidly in concentrations below 25 per cent than it does in those above. Spermatozoa from the different fish differed in absolute survival time, but the relative survival times were similar.

II. ACTIVITY IN ARTIFICIAL SEA WATER

In artificial sea water (Fig. 1) the spermatozoa behaved about the same as they did in true sea water. The spermatozoa showed first signs of motility at a concentration of 15 per cent artificial sea water. In one sample they lived as long in 20 as they did in 25 per cent concentration. These were minor discrepancies. The artificial sea water was used to avoid the rather large and irregular variations in pH. In the true sea-water concentrations ranging from 15 to 200 per cent, the pH varied as follows: 7.8, 7.8, 8.0, 8.0, 7.8, 9.1, 8.9, 8.5. In the artificial sea-water concentrations grading from 15 to 150 per cent, the values were: 8.4, 8.0, 7.95, 7.9, 7.95, 8.3.

Spermatozoa which had been inactivated in 10 per cent artificial sea water became active when the solution was brought to a higher concentration. Those which had been in fresh aquarium or distilled water for a few minutes, however, showed no signs of motility when the water was brought to a concentration as high as 100 per cent osmotic pressure of sea water. Spermatozoa which in time had become inactive in 150 per cent artificial sea water were not reactivated by dilution.

III. ACTIVITY IN NONELECTROLYTES

In an effort to determine whether the observed differences in spermatozoan activity at different salinities were due to the effects of the particular ions in sea water or merely to the osmotic pressure, solutions of glucose with the same osmotic pressure as those of the sea water were tested. The spermatozoa were not activated below concentrations of 50 per cent. At this concentration the sperma-

tozoa were motile for a longer period than in higher concentrations (Fig. 2). It seemed likely that in glucose, as compared with sea water, there was a difference in permeability. In an attempt to provide a medium to which the cells were less permeable, solutions of sucrose were made with $CaCl_2$ added. Here again the optimum solution was at 50 per cent of the osmotic pressure of sea water (Fig. 2). In one trial the spermatozoa were motile in a solution as low as 20 per cent. In the solutions of both sugars the results were not so uniform as in the artificial and true sea waters. Furthermore, the activity was less protracted.

IV. EFFECT OF pH

A few tests were undertaken to check the possibility that differences in pH may have affected the results recorded above. Solutions of 50 per cent artificial sea water were titrated with HCl or NaOH to a pH of 4.8, 6.05, 7.1, 7.55, 8.25, 9.25, and 10.15. The extremes of this series well exceeded the pH of any of the solutions tested in the previous experiments. There was no noticeable difference of

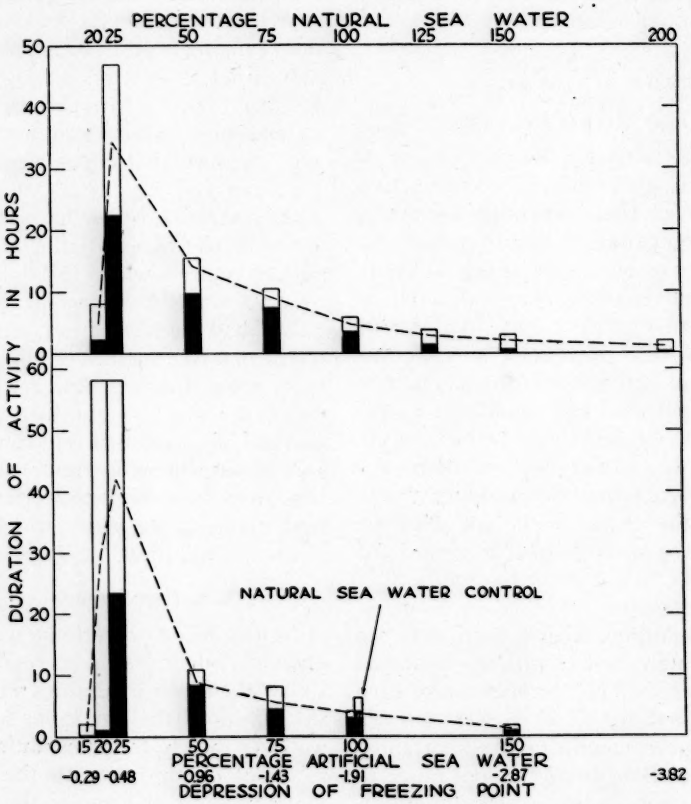
PERCENTAGE NATURAL SEA WATER
20 25 50 75 100 125 150 200
DURATION OF ACTIVITY IN HOURS
NATURAL SEA WATER CONTROL
0 15 20 25 50 75 100 150
PERCENTAGE ARTIFICIAL SEA WATER
-0.29 -0.48 -0.96 -1.43 -1.91 -2.87 -3.82
DEPRESSION OF FREEZING POINT

FIG. 1.—Survival of goby spermatozoa in different concentrations of true and artificial sea water. Total height of column equals maximum survival time in four experiments, two slides for each experiment. Height of dark area equals minimal survival time. The curve is plotted on points which are average survival times for the different trials in each solution. Temperature 23°–26° C.

behavior of the spermatozoa in the different pH concentrations. They all became inactive at practically the same time.

V. PROLONGATION OF ACTIVITY AT LOW TEMPERATURE

Temperature, on the other hand, had a remarkable effect on the period of spermatozoan motility. The spermatozoa from one fish were placed in the various concentrations of artificial sea water. One set of the slides was kept in a refrigerator at a temperature of 2°–4° C. A similar set from the same testes was retained at room temperature, 24°–26° C. The spermatozoa in 17 per cent solution became inactive at the same time; but in all the higher concentrations the spermatozoa lived for a much greater length of time at the lower temperature (Fig. 3). Those in 20 and 25 per cent showed activity for over 2 weeks as compared to 33 hours for those kept at room temperature in the same solutions. It is presumed that they were in motion throughout this long period, for they showed activity when the slide was placed under the microscope immediately after removal from the refrigerator.

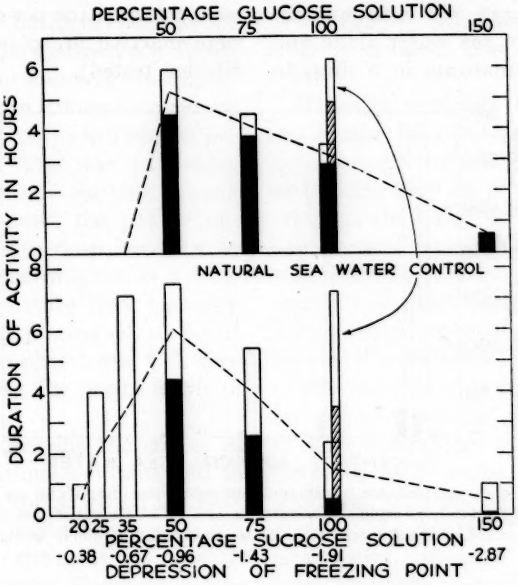

FIG. 2.—Survival of goby spermatozoa in glucose and sucrose solutions osmotically equivalent to the sea-water solutions. $CaCl_2$ added to the sucrose. Total height of column equals maximum survival time in two experiments, two slides for each experiment. Height of dark column equals minimal survival time. Temperature 24°–26° C.

VI. RELATION OF "SEMINAL VESICLE" TO ACTIVITY OF SPERMATOZOA

The gobies are among the few teleosts that possess a well-defined "seminal vesicle." Some attention was given to the possible relations of the "seminal vesicle" to the duration of activity of the spermatozoa of *Gillichthys*, since Eggert (1931) claimed that the fluid extracted from the vesicles of a related species, *Gobius panizzae*, seemed to prolong the life of the sperm. Neither in origin nor in structure is this organ closely similar to the

seminal vesicle of mammals. It is primarily a secretory organ (unpublished personal observations), but its physiological function has not been determined.

That no prolongation of spermatozoan activity can be attributed to the vesicular extracts of *Gillichthys* was indicated by Young and Fox (1937) and is confirmed by the present study. Twelve slides were prepared, six with spermatozoa in a drop of sea water alone and six with the spermatozoa in a drop to which vesicular fluid had been added. The movements on each slide ceased within a few minutes of one another. There was no significant difference in the length of activity.

Vesicular fluid was also tried with lower concentrations of sea water. No visible prolongation of the life of the spermatozoa could be noticed, nor were they active below concentrations of 18 per cent. Observations were not continued to the termination of the experiments at all the dilutions. It was considered that any differences observed could have been caused by the changes in osmotic pressure that resulted from the addition of the vesicular fluid.

VII. MISCELLANEOUS OBSERVATIONS

The fish used in all these tests were kept without food, some for as long as 4 weeks. Inanition had no effect on the motility of the spermatozoa, nor did it reduce the period of activity.

Three gobies were placed in fresh water for 2 weeks before their testes were removed. Spermatozoa from these fish were active in 100 per cent sea water but were inactive in 10 per cent (the only dilution tested).

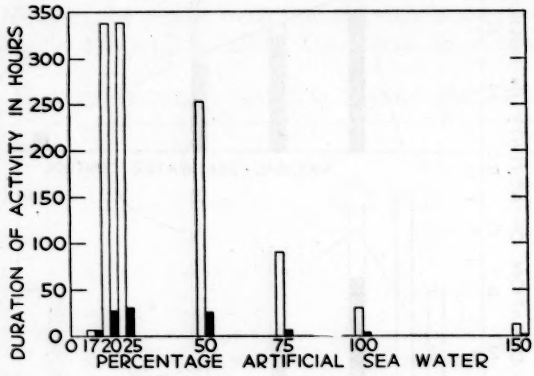

FIG. 3.—Survival of goby spermatozoa in different concentrations of artificial sea water and at different temperatures. Height of light column equals survival time at 2°–4° C. Height of dark column equals survival time at 24°–26° C. A control in 100 per cent true sea water reacted similarly to that in the 100 per cent artificial sea water.

DISCUSSION

The methods used in the experiments did not lead to very precise determinations. With the addition of spermatozoa to the solutions, there was an unavoidable addition of materials which must have changed the osmotic pressure from that calculated. The gaps between the percentages of the solutions tested did not permit the determination of an exact optimal percentage. Since the teased spermatozoan preparations contained ions, the sugar solutions were not truly nonelectrolytic after the spermatozoa had been introduced. Also the number of spermatozoa in each preparation varied.

Since low temperatures markedly pro-

longed the life of the spermatozoa, no doubt the failure to control the temperature closely in the exeriments introduced an error. This error was probably not large, however, for the temperature differences were limited and all the experiments were subjected to approximately the same fluctuations (see explanation of figures).

Within the wide limits between which they were tested, the spermatozoa do not appear affected by the pH concentrations. For this reason it may be assumed that no serious error was introduced because of the fact that the pH value varied in the different solutions. Young and Fox (1937) found the pH of unbuffered sea water to drop from 8.1 to about 6.3 during the progress of similar experiments. They state that buffering the sea water and placing N/10 KOH in the slide well to absorb the CO_2 generated did not affect the length of life of the spermatozoa.

Attempts to determine the degree of activity in the various solutions and to correlate this with the length of life were unsuccessful. The movements were obviously reduced in the solutions of minimum osmotic pressure. However, it was in these solutions that the time of motility was shortest. In all the solutions of higher pressures the spermatozoa seemed to have equal activity.

In spite of these limitations, I believe the experiments allow one to arrive at certain generalizations.

The spermatozoa of the long-jawed goby are active over a surprising range of osmotic pressure. If the delta of the sea water is taken as $-1.°91$, this range extends from $\Delta = -3.°82$ to $\Delta = -0.°33$. To my knowledge, no other teleost spermatozoa investigated are active over such a wide range. This wide tolerance is possibly connected with the life-habits of *Gillichthys*. The fish themselves are capable of living for some weeks both in fresh water and in supersaturated sea water (personal observations). It is also likely that they spawn in sea water of different concentrations.

At $\Delta = -0.°48$ (25 per cent) sea water and artificial sea water, the spermatozoa live for a much longer time than at pressures above or below this. However, in nonelectrolytic solutions the longevity is greatest around $\Delta = -0.°96$ (50 per cent).

It would naturally be assumed that the spermatozoa live longest in solutions isotonic with the fish body fluids. In all probability they are in osmotic equilibrium with the blood of the fish. Davson and Danielli (1943) find the eggs of salmon and trout to have the same osmotic pressure as the female's blood, and Krogh (1939) states the same to be true for two marine teleosts.

The blood of marine fishes has a definitely higher osmotic pressure than that of fresh-water fishes and of anadromous forms in fresh water. The average for seventeen different salt-water species is $\Delta = -0.°84$, whereas the average for six fresh-water fish is $\Delta = -0.°54$ (tables from Mathews, 1920; Davson and Danielli, 1943; and Heilbrunn, 1943). When taken on their fresh-water spawning grounds, the serum of the male Pacific salmon has a mean of $-0.°668$ and the ovarian fluid of $-0.°559$ (Greene, 1904).

It is apparent that the blood of marine teleosts has a much higher osmotic pressure than do the dilutions of sea water, true and artificial, in which the goby spermatozoa live the longest. The reaction of the spermatozoa in the nonelectrolytic solutions more nearly parallels what might be expected. The sugar solution which provides the maximum period of activity has a pressure of $\Delta = -0.°96$. This is much closer to osmotic equilibrium with the blood of the fish (Table 1).

Although the salmon (*Salmo salar*) spends most of its adult life in the ocean, it develops in fresh water, and it migrates to fresh water to spawn. Its spermatozoa live a much longer time in dilutions of sea water than they do in river water. The duration of activity is greatest in sea water diluted to $\Delta = -0.^{\circ}38$ (20 per cent). In sucrose solutions the activity is longest in a solution of $\Delta = -0.^{\circ}48$ (25 per cent of sea water) (Ellis and Jones, 1939). These are both somewhat lower than the probable osmotic pressure of the blood. As in the goby, the optimum sugar solution has a higher pressure than the optimum sea-water dilution. The difference, however, is much less.

TABLE 1

RELATION OF OSMOTIC PRESSURE OF THE BLOOD TO SOLUTIONS OPTIMUM FOR SPERMATOZOAN ACTIVITY

	Approximate Osmotic Pressure of Blood	Osmotic Pressure of Solution for Optimum Activity of Spermatozoa	
Salmon (fresh water)	$\Delta = -0.^{\circ}60$	Sucrose	$\Delta = -0.^{\circ}48$
		Sea water	$\Delta = -0.^{\circ}38$
Goby (salt water)	$\Delta = -0.^{\circ}84$	Sucrose and glucose	$\Delta = -0.^{\circ}96$
		Sea water	$\Delta = -0.^{\circ}48$

The osmotic pressure of a solution in relation to a membrane is not dependent wholly on the concentration of the solution. The pressure decreases in proportion to the ease with which the substances pass through the membrane. That there is a difference for the optimum concentrations of sea water as compared with sugar solutions indicates that we are here dealing with such a condition. It is possible that there is an outward penetration of substances from the spermatozoa in isosmotic solutions of sea water. Solutions of sea water in which spermatozoa survive longest have a markedly lower osmotic pressure than does the blood of the fish. Optimum sugar solutions are more nearly isosmotic.

Ellis and Jones (1939) believe that the activating effect of sea water is osmotic rather than ionic. Their salmon spermatozoa lived slightly longer in sea-water dilutions than they did in sugar solutions; but Ellis and Jones did not believe that they had undertaken enough experiments to make the finding significant. In all my investigations the goby spermatozoa lived much longer in the sea water, true and artificial, than in either of the sugars. Though the osmotic factor is a major one, my data indicate that the ions also play an important role. The sea-water dilutions are a better medium for the spermatozoa than are osmotically identical sugar solutions.

The spermatozoa of the salmon and the long-jawed goby are longest active at nearly the same concentration of diluted sea water. The same is probably true also of the spermatozoa of the trout (in his short note, Huxley [1930] states that trout spermatozoa lived longest in sea water diluted to 25 per cent with tap water). Yet this goby spawns in sea water or in somewhat diluted sea water, whereas the salmon spawns in fresh water. There is, however, a marked adaptation in both cases to the environment in which they spawn. The salmon spermatozoa are active, though for a short time, in fresh water and are inactive in concentrations above 40 per cent sea water. The goby spermatozoa are totally inactive in fresh water, become active at about 17 per cent sea water, and maintain motility even in supersaturated sea water. The spermatozoa of the goby are much better

adapted to their natural medium than are those of the salmon. The fact that the bays and estuaries inhabited by *Gillichthys* are subject to an influx of fresh water during the period when this goby spawns may be related to the fact that the spermatozoa are longest active at a reduced salinity. Determinations for other species, however, are needed to verify this possibility. It would be interesting to make comparisons with fishes that are permanently resident in fresh water and with oceanic fish that are not estuarine dwellers.

For the survival of the species, there would probably be no added advantage for the goby spermatozoa to live a longer time than they do in their natural spawning medium. The spermatozoa can live at least 3 hours in sea water at usual temperatures. In this time they must certainly be capable of fertilizing the eggs. The goby males and females spawn practically simultaneously and in close proximity (Weisel, 1947). Since the spermatozoa become active more quickly in straight sea water, it is actually more advantageous for the spawning to occur in this medium rather than in the solution for optimum survival time. The lag of 2–5 minutes in activation of the spermatozoa in 25 per cent sea water might lead to failure in fertilization.

Obviously, the fluid from the "seminal vesicle" aids little, if any, in propagation of the species by prolonging the life of the spermatozoa as some have suggested. Its function is obscure.

Unlike the trout (Huxley, 1930), the long-jawed goby is adapted to its normal environment not only somatically but also for reproduction.

There appears to be no adaptational significance and perhaps no historical significance to the ability of the *Gillichthys* spermatozoa to remain active for many days at low temperature. This ability may merely reflect the general rule that low temperatures retard the rate of biological processes.

SUMMARY

1. The spermatozoa of the long-jawed goby are active over a wide range of osmotic pressure—from 17 to 200 per cent sea water. They are motile longest in 25 per cent sea water and in glucose and sucrose solutions that have the same osmotic pressure as 50 per cent sea water. The optimum activity in 25 per cent sea water may be related to the fact that the salinity in the goby's habitat is often reduced during the spawning season.

2. The concentration of true and of artificial sea water at which the spermatozoa live longest is not isosmotic to the spermatozoa. Optimum sugar solutions are more nearly isosmotic.

3. The spermatozoa live longer in various concentrations of sea water than in osmotically equivalent sugar solutions. Though the activating influence of the sea water may be largely osmotic, the ions also appear to play an important role.

4. A lower temperature markedly prolongs the life of the spermatozoa in the different concentrations of artificial sea water. In 25 per cent concentration at 2°–4° C. the spermatozoa remained alive for 2 weeks, whereas controls at 24°–26° were immobile in 33 hours. The prolonged activity of the spermatozoa at low temperatures probably has no adaptive significance.

5. Fluid from the goby's "seminal vesicle" does not prolong the life of the spermatozoa. The function of the vesicle remains obscure.

6. The spermatozoa of the fresh-water-spawning salmon and the salt-water-spawning goby survive longest in sea-water dilutions of about the same osmotic pressure. In spite of this similarity

the activity of the spermatozoa appears to differ in correlation with their respective environments. The spermatozoa of the goby are inactive in fresh water but are active in undiluted sea water, whereas those of the salmon are active in fresh water but are inactive in normal sea water.

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THE TEMPERATURE COEFFICIENTS OF DDT ACTION IN INSECTS[1,2]

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With a variety of insect species and methods of application, it has been found by various workers that DDT—2,2-bis(p-chlorophenyl)1,1,1-trichlorethane—can be more toxic at lower temperatures than at higher temperatures. Lindquist *et al.* (1945) first recorded that adult houseflies, *Musca domestica*, treated with residual-type sprays of DDT were knocked down and killed faster at lower than at higher temperatures. Later, Lindquist *et al.* (1946) reported quicker kill with DDT residual sprays for adult mosquitoes, *Anopheles quadrimaculatus* and *Culex quinquefasciatus*, and bedbugs, *Cimex lectularius* and *C. hemipterus*. Potter and Gillham (1946) obtained a higher mortality of adult grain beetles, *Tribolium castaneum*, at lower temperatures after spraying with DDT solutions in oil. And Richards and Cutkomp (1946) likewise obtained a greater kill of aquatic mosquito larvae, *Aedes aegypti*, and shrimp, *Gammarus* sp., at lower temperatures on immersion in very dilute suspensions of DDT.

In view of the fact that a negative temperature coefficient such as that reported by these workers has seldom been

[1] Paper No. 2291 ("Scientific Journal Series" [St. Paul 1, Minn.: Minnesota Agricultural Experiment Station, 1947]).

[2] The work described in this paper was begun under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the University of Minnesota. It was completed under a contract between the Medical Division of the Chemical Warfare Service and the University of Minnesota.

recorded in the field of biology, the following studies were undertaken in an effort to determine (*a*) more about the manner in which the toxicity of DDT varies with temperature and (*b*) the nature of the reactions involved. The data obtained are consistent with the hypothesis of cuticular accumulation by adsorption advanced by Richards and Cutkomp (1946), but there are other possible explanations. Satisfactory elucidation of these peculiar results must await more conclusive evidence.

MATERIALS AND METHODS

Larvae of the mosquito *Aedes aegypti* and a midge *Chaoborus* sp. were used as the principal experimental animals. The larvae of *Aedes* were reared in the laboratory at a constant temperature, 30° C., except in one case noted otherwise in text. Yeast was used as food for the first instar larvae, then whole-wheat bread was used for later stages. Late third-instar larvae were used for the tests. In order to obtain a uniform population, simultaneous hatching of the eggs was induced by lowering the oxygen tension by pulling a partial vacuum over the eggs (see Gjullin *et al.*, 1941). The larvae of *Chaoborus* were collected from a little pond near by in the middle of the fall of 1945 (October 10–November 6) at a time when the field temperatures varied from 5° to 20° C. These larvae were full grown, of constant physiological age, and just about ready to hibernate. Immediately after being collected, they were placed in a cabinet at 5° C. for a few days before being used.

Vials 1 inch in diameter and 3 inches in height were used as the testing vessels. About 15 ml. of a DDT suspension of a known concentration[3] was poured into each vial. Ten larvae were introduced into each vial, which was then stoppered to prevent evaporation during the test period. Control tests (acetone solution without DDT) were always run. Sublethal concentrations of DDT suspensions were also a kind of control. Checks on the retention of toxicity of suspensions were made by subsequent bioassay, and the suspensions were further controlled by renewal daily. Four replicates, each with ten larvae, were used in each set of conditions for each test. Each test was repeated at least once and in most cases more than once. Accordingly, each curve in Figures 1–8 is based on eighty or more test animals.

In an attempt to obtain an experiment that might approach being a critical test, larvae were pretreated at one temperature in a DDT suspension and then transferred to another temperature. For these experiments, six sets of vials containing the desired concentrations of DDT and numbers of larvae were made up; three of the sets were placed in a low-temperature cabinet, one set remaining there during the whole experimental period, a second set being shifted to a high temperature at the end of 12 hours, and the third set being shifted to a high temperature at the end of 24 hours; the other three sets were placed in a high-temperature cabinet and shifted to low temperatures in a similar manner. Those sets of vials remaining at low or high temperatures throughout the experimental period were used as controls for those which were shifted from high to low or low to high temperatures, respectively. Four replicates were used in each of these tests also, and the experiment with *Aedes* was also repeated; but the experiment with *Chaoborus* could not be repeated because of scarcity of specimens.

In order to interpret the immersion

[3] The DDT suspensions were made by diluting a stock solution containing 0.1 gm. of DDT in 100 ml. of acetone. Distilled water was used for the test solutions.

data more satisfactorily, parallel injection experiments were made using *A. aegypti* larvae. These larvae were injected with a microsyringe through the lateral abdominal wall using the emulsion formula developed by Roeder and Weiant (1946). The larvae used were in the range of 4–5-mg. live weight, and the volume of injection was kept constant at 0.25 cu. mm. Injections were made at room temperatures, and the larvae were then placed in vials of water at different temperatures and subsequently handled in a manner comparable to that used for the immersion tests. With the injection experiments there was naturally a considerable mortality among the control animals. No correction is made for this on the graphs; instead, curves are given for mortality of both experimental and control animals.

Readings on percentage of mortality in the immersion tests were taken every 12 hours for a period of 5 days; readings in the injection experiments were made twice daily but at less regular intervals, as shown by the points on the graphs. The criterion of death chosen was the absence of visible movement under a magnifying glass for a length of time of approximately 1 minute. Living larvae are quite active and even when dying normally make feeble movements every few seconds.

Curves were all drawn freehand through the points obtained, and no statistical analysis was performed because the work presented is essentially qualitative. Some variation naturally occurred between the various repetitions, but the same sequence of curves was maintained in each test.

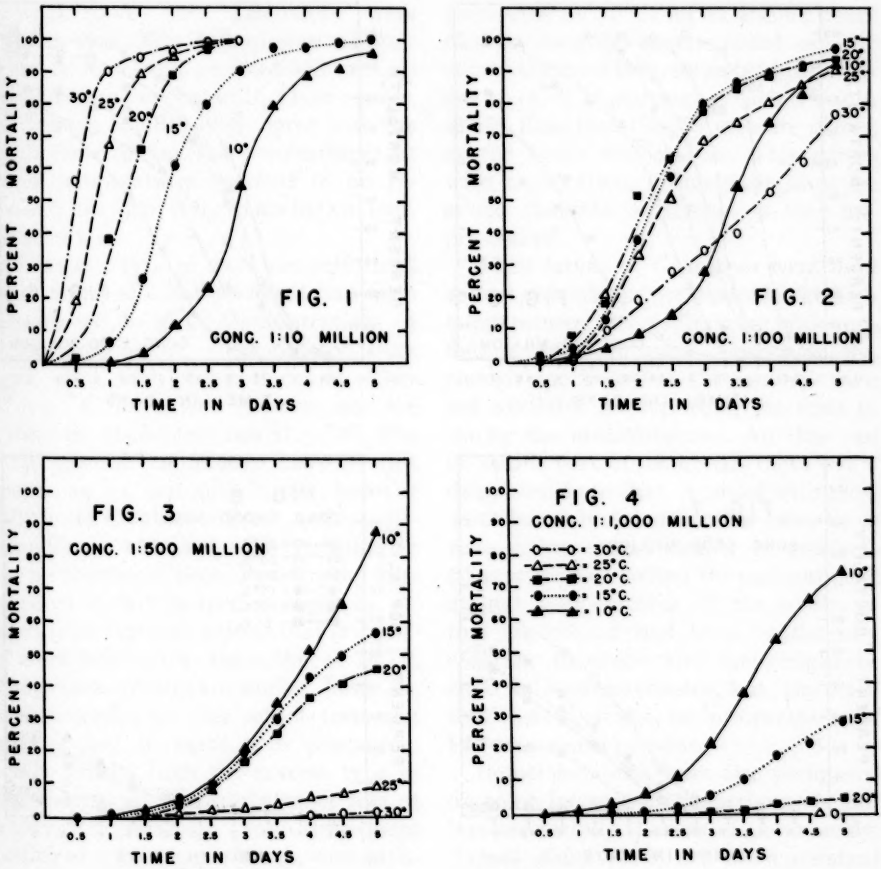
IMMERSION TESTS

The data are presented in the form of graphs (Figs. 1–10). The results obtained with the different concentrations of DDT with the larvae of *A. aegypti* at different temperatures are shown in Figures 1–4. In Figure 1 the curves show clearly that at a concentration of DDT of 1 part in 10,000,000, the mortality of the larvae was accelerated by a rise in temperature, i.e., there is a positive temperature coefficient at this concentration. When the concentration of DDT was lowered to 1 part in 100,000,000, as shown in Figure 2, the mortality at 20°, 25°, and 30° C. dropped considerably. As the concentration of DDT was lowered further to 1 part in 500,000,000 or below, as shown in Figures 3–4, an entirely reversed situation was observed: the mortality of the larvae decreased with a rise of temperature, i.e., there is a change to a predominating negative temperature coefficient. It should be noted that there is no indication of a temperature optimum for dilute concentrations within the range of temperatures studied; the data show only an increase in effectiveness with decreasing temperature.

The mortality of the controls which contained no DDT but the maximal amount of acetone which might be in the DDT suspensions was negligibly low, except when the concentration of acetone reached 0.01 per cent (corresponding to the acetone concentration in a suspension of DDT of 1 part in 10,000,000). In the case of this high acetone concentration, a mortality of approximately 20 per cent was observed at the end of 5 days in the case of animals held at 10° C. The other temperatures were not particularly affected by any of the acetone concentrations used. The only effect, then, of correcting for the mortality of controls would be to increase slightly the size of the positive temperature coefficient already noted for this concentration of DDT, since in these controls the kill was significant only at the lowest temperature.

The results obtained with the different concentrations of DDT with the larvae of *Chaoborus* sp. are shown in Figures 5–8. These data show a very similar, but more gradual, change than in the case of the larvae of *A. aegypti*. Again, lowering the temperature results in an increasingly higher *relative* kill as the concentrations of DDT are progressively decreased. Particularly striking in these four sets of graphs is the fact that, first, the highest temperature, then the next highest, and then the third highest decrease in effectiveness with decreasing concentration. The mortality of controls for the *Chaoborus* tests was nearly zero at all temperatures.

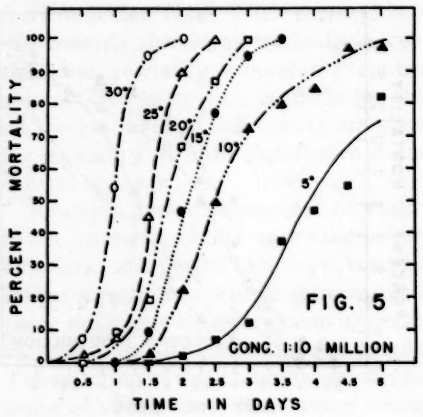
If adsorptive processes are acting as a concentrating mechanism or if low temperature is, by some other means, directly facilitating the entry of action of DDT, one might expect that pretreatment in DDT suspensions at low temperatures would augment mortality during subsequent testing at higher temperatures. And, conversely, pretreatment in

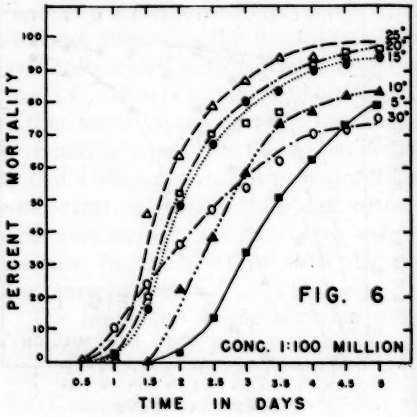


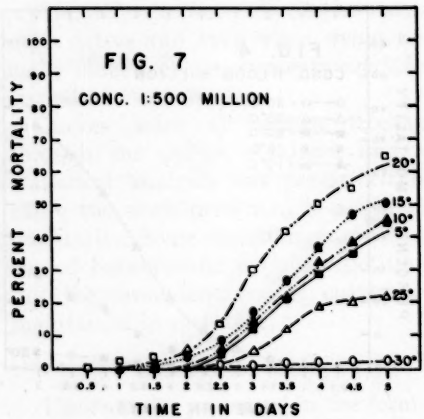
FIGS. 1–4.—Mortality curves for larvae of *Aedes aegypti* at different temperatures and different concentrations of DDT suspensions as labeled.

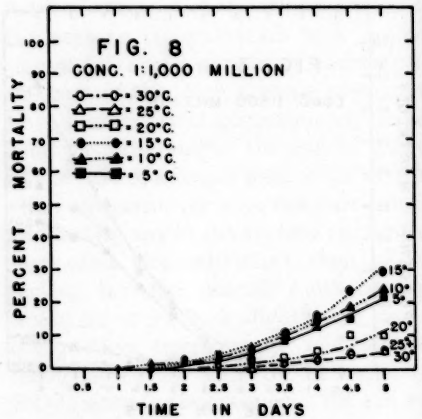
DDT suspensions at higher temperatures should have little or no effect on the animals, with the result that transfer to lower temperatures would be comparable to starting the test at the time of transfer to the lower temperature. The results obtained from pretreatment at low or high temperatures in dilute concentrations of DDT are in general agreement with these predictions, but in one case discussed below (pretreatment of *Chaoborus* at high temperatures) the results were erratic for some unknown reason. Conceivably, these data might be accounted for by any mechanism for enhancing the effect of low concentrations by lowering the temperature. Accordingly, the data support only the reality of the negative temperature coefficient component.

Replicated tests with two different groups of larvae of *A. aegypti* (in this case reared at 25° C.), pretreated in DDT suspensions of 1:100,000,000 and 1:500,000,000 at 15° C. for 12 and 24 hours and then changed to 30° C., gave









FIGS. 5–8.—Mortality curves for larvae of *Chaoborus* sp. at different temperatures and different concentrations of DDT suspensions as labeled.

consistent results but not very smooth curves (perhaps because of insufficient data). At a concentration of 1:100,000,000 there was no significant difference between test groups held throughout at 30° C. and those given only a 12-hour pretreatment at 15° C.; but there was a greater kill following a 24-hour pretreatment, and this difference was evident throughout the term of the experiment (87.5 per cent kill in comparison to 76.9 per cent kill in groups held at the higher temperature throughout the experiment). At the lower concentration, 1:500,000,000, the differences were greater, especially following a 24-hour pretreatment (27.5 per cent kill in comparison to 12 per cent kill). These curves, though not smooth lines, agree with the prediction in that the pretreatment at lower temperatures resulted in an increased kill *after* transfer to higher temperatures.

The same type of tests was performed with larvae of *Chaoborus* that had been accustomed to 5° C. Concentrations of 1:100,000,000 and 1:500,000,000 were again used, with pretreatment at either 5° or 10° C. for 12 and 24 hours, and the remainder of the test run at 30° C. The same general relationship held for this species as for mosquito larvae, but the differences were not so great, and less difference was obtained with variation in the pretreatment time. Perhaps the lesser effect with this species might be related to its being an animal that is slightly more resistant to the action of DDT. The general results are similar, however, and, accordingly, this set of tests can also be said to agree with prediction.

The results from the reverse type of experiment, where pretreatment was at higher temperatures and subsequent testing at lower temperatures, were satisfactory with only one of the two species. One would expect little or no effect from the period of pretreatment at higher temperature and, accordingly, that the mortality curves would lag behind those for test groups, which were held throughout the experiment at lower temperatures by a period roughly corresponding to the length of the pretreatment period. When larvae of *A. aegypti* were used as test animals, the data agree fairly well with expectation, provided that correction is made for mortality that may be attributed to temperature effects (as shown by controls without DDT). With this correction, the mortality curves for series pretreated at 35° C. for 12 and 24 hours can be roughly superimposed on the curves obtained from series held throughout at 15° C. if plotting for each is begun at the time that the cultures are placed at the lower temperature. This agrees with expectation, although it must be added that the agreement is only approximate.

When larvae of *Chaoborus* were used as test animals for pretreatment at high temperatures, the results were both conflicting and confusing. Unfortunately, enough specimens of this animal were not available for repeating the tests to clarify the inconsistencies. All that can be said is that in some vials there was a delay similar to that obtained with mosquito larvae but that in other cases there were either excess delays or accelerations of mortality from the pretreatment at high temperatures. If the results of this experiment had been consistently contrary to prediction, they might be cited as counterevidence, but, since the results were erratic, no interpretation of the data seems possible.

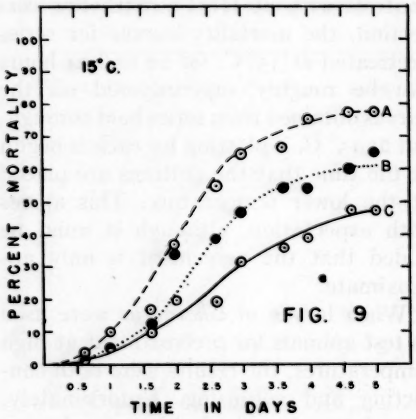
Immersion tests were also performed on some larger aquatic arthropods but less successfully. Larvae of the cranefly, *Tipula abdominalis*, are more resistant to DDT than are those of *Aedes* and *Chaoborus*. Not all larvae die, even in

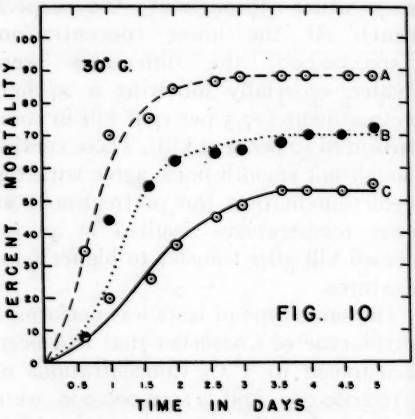
concentrations of 1 part per million. At this concentration a positive temperature coefficient is clearly shown. At somewhat lower concentrations (1 part in 5,000,000 or 10,000,000) there is little mortality at either high or low temperatures, but it can be noted that many or all of those at the lower temperatures exhibit the symptoms of DDT-poisoning, whereas those at the higher temperatures do not. Immersion tests using crayfish, *Cambarus* sp., were completely unsatisfactory, owing, at least in part, to inadequate facilities for handling large numbers of these animals in our laboratory; mortality among controls at higher temperatures precludes use of the data for comparison of temperature effects. The work with crayfish is mentioned only to say that, like cranefly larvae, they tolerate relatively high concentrations of DDT. Several other arthropods are more or less resistant to DDT, as has been shown by the field work of numerous individuals.

INJECTION TESTS

If the arthropod cuticle plays an important causal role in these manifestations of DDT toxicity at different temperatures, one might expect to obtain different results if the role of the cuticle could be circumvented by direct injection into the blood stream. On the other hand, if the same phenomenon of shift from positive to negative temperature coefficient is encountered with decreasing dosage after injection, then the phenomenon might be due to competing internal phenomena such as the toxic action versus a detoxifying mechanism.

Injections were made into mosquito larvae in the weight range of 4–5 mg. live weight, using a constant injection volume and varying the dosage by varying the concentration of DDT in the emulsion. Data are presented (figs. 9–10) only for near-minimal dosages, since it is in this range that the shift to a negative temperature coefficient would be expected to occur. The data show that the





FIGS. 9–10.—Mortality curves for larvae of *Aedes aegypti* injected with DDT emulsions. Larvae in range of 4–5-mg. live weight, injected with 0.25 mm.^{3} of emulsions containing 0.02 mg. DDT per ml., 0.01 mg. DDT per ml. and 0.00 DDT per ml. (control). Curve *A* in each graph based on 58 larvae, each of which received a dose of approximately 1.0 γ/g.; curve *B* in each graph based on 61 larvae, each of which received approximately 0.5 γ/g.; and curve *C* in each graph based on 64 larvae receiving the emulsion without any DDT.

doses are indeed near minimal and that the temperature coefficient remains positive.

Similar injection data were also obtained for several larger species of arthropods for which we do not have satisfactory parallel immersion data. Injections of approximately four hundred cranefly larvae, *T. abdominalis*, showed a clear positive temperature coefficient for toxicity. On a 5-day basis, the median lethal dose was considerably below 10 γ/gm at 20° C. but over 10 γ/gm at 5° C. Higher doses gave more rapid kill but agreed with lower doses in always showing a positive temperature coefficient. With crayfish, *Cambarus* sp., 483 injections showed the median lethal dose on a 5-day basis to be in the range of 0.5 γ/gm at 20° C., whereas it was nearly 10 γ/gm at 3° C.

With three different species, then, we have consistently obtained a positive temperature coefficient for DDT action after injection, irrespective of dosage. Since the shift to a negative temperature coefficient at low dosages occurs only when the DDT is applied externally, the phenomenon is not to be interpreted in terms of competing internal processes or hypothetical developmental optima. The results can be explained only in terms of absorption through the cuticle.

DISCUSSION

As a result of their preliminary experiments, Richards and Cutkomp (1946) suggested that the cuticle of arthropods (insects, etc.) acts as a concentrating device which accumulates DDT from dilute solutions and suspensions by adsorption and somehow makes it available to the internal tissues. This hypothesis was based on five points. (1) A much greater effect from external applications in those animal groups which possess a chitin-protein cuticle than in those without cuticles of this type. Injection of emulsions gives relatively similar results with different animals, whereas external applications give very different results. This was taken to indicate an important role for absorption and to implicate the arthropod exoskeleton as a possible intermediary—a view which has subsequently been expressed also by Tobias *et al.* (1946*a*). (2) Preliminary temperature studies indicated a negative temperature coefficient under certain conditions. (3) Solubility tests showed that DDT has a positive temperature coefficient for solubility in both polar and apolar solvents. (4) Direct adsorption tests, with subsequent DDT determinations by bioassay method, showed that DDT could be adsorbed from suspensions by chitinous cuticles (and activated charcoal) but not by several other types of tissues or body coverings. And (5) negative evidence against the possibility that DDT forms some more toxic metabolite was summarized from the literature. The present work was undertaken to study further the rather peculiar situation of a shift in temperature coefficient from positive to negative and to attempt to evaluate the above hypothesis.

The adsorption-concentrating hypothesis was advanced to interpret the different susceptibilities of different animal groups to the external application of DDT. The actual mode of toxic action of DDT to protoplasm is a separate problem (Richards and Cutkomp, 1946) for which a preliminary interpretation may be made on the basis of data published by Yeager and Munson (1945), Roeder and Weiant (1946), Merrill *et al.* (1946), Tobias and Kollros (1946), Tobias *et al.* (1946*b*), and others. Obviously, no single point can explain all the facets of DDT action. Clearly, several processes are necessary to explain the existence of both positive and negative temperature co-

efficients for external application when only a positive temperature coefficient is obtained following injection. Since the phenomenon of a negative temperature coefficient is encountered only when the arthropod body wall is a barrier between the DDT and the living protoplasm, this negative temperature coefficient component must be concerned with the body wall or exoskeleton. Since the present paper is concerned with the negative temperature coefficient and its interpretation, we do not need to consider here the effects of DDT on living protoplasm. Readers are referred to the papers cited above for a presentation of the effects of DDT on nerves and other tissues.

The full story of the forces responsible for the entry of DDT into animals and the details of the route traversed is too complex for analysis by the gross methods employed in the present study. The data obtained do show, at least qualitatively, the following facts:

1. One can obtain either a positive or a negative temperature coefficient, depending on the conditions of the experiment.

2. The negative temperature coefficient is obtained only from external applications. Therefore, it must be related to absorption through the body wall and is not explainable in terms of detoxification, excretion, or other internal phenomena. Nor is it to be explained, as Potter and Gillham (1946) attempt, by speculations about developmental optima.

3. The negative temperature coefficient is obtained only with low concentrations or low spray dosages, i.e., only when concentration or accumulation of the toxin might be expected to be of significance (as shown by comparison of minimal lethal doses for injection with minimum lethal concentrations for immersion). Since it has been shown (Richards and Cutkomp, 1946) that arthropod cuticle can remove DDT from suspensions, whereas living tissues do not do so to any readily demonstrable degree, and since adsorption is favored by low temperatures, it seems to us possible that the arthropod cuticle (rather than the entire body wall) is responsible for the observed phenomena and that it may act in part by adsorption processes. One has to remember, however, that we are dealing with colloidal suspensions, not true solutions and that, accordingly, the applicability of the term "adsorption" is open to question. It is conceivable that the colloidal particles might spread on cuticular surfaces in a manner similar to that analyzed by Volmer (1932, 1938), whereas the colloidal particles probably would not be capable of passing through the cuticular pores. Also we cannot rule out the possibility of solid solution of DDT. In any event, we have to admit that analytical chemical methods do not permit direct demonstration of the postulated accumulation and that the available data do not permit of a convincing picture of the mechanics of the cuticle acting as a concentrating organ.

Alternatively, it is conceivable that the cuticle does not act as a *concentrating* organ but only as a layer, readily penetrated by DDT, whose inner surface (cuticle-cell surface) either readily receives DDT in a way naked cells do not or whose inner surface brings the DDT into contact with specially sensitive cell parts that are only ineffectively reached by injections into the blood stream. Such a hypothesis would necessitate assuming that the cuticle is either more penetrable at lower temperatures (by membrane change or by adsorption forces) or that the underlying receptors are less resistant. The possibilities outlined in this paragraph are purely hypothetical, but the available data do not eliminate them.

4. Variations in effectiveness of DDT

between different species of arthropods show that mere possession of a chitin-protein cuticle does not guarantee high sensitivity to external applications of DDT. But it is well known that arthropod cuticles are heterogeneous systems which vary a great deal in chemistry, structure, thickness, and permeability. The cuticle is too complex and too little understood as yet to make it profitable to speculate about the variations in DDT-sensitivity being caused by variations in cuticle composition, although it is conceivable that this is the case. Whatever the explanation of these data may be, certainly we must conclude that some arthropod cuticles are more efficient receivers of DDT than are others.

5. Obviously, the negative temperature coefficient at low concentrations of DDT must be due to properties of the body wall of the arthropod, probably of the cuticle itself. The reason for this might be that the cuticle concentrates DDT by adsorption processes or that it becomes truly more permeable at lower temperatures or that hypothetical receptive surfaces at the cuticle-cell interface have their resistance to DDT greatly lowered. The available data do not permit selecting between these alternatives, but the authors are inclined to favor the idea that the cuticle acts as a concentrating organ.

To conclude this part of the discussion, if one is willing to make the necessary assumptions, these data can be explained on the cuticle-concentrating hypothesis of Richards and Cutkomp. For this, one has to assume that there is a necessary threshold concentration; that no further concentrating is necessary at the higher concentrations employed but that at lower concentrations further concentrating is necessary; that colloidal DDT particles will somehow spread on surfaces in the cuticle matrix and that surface diffusion of the adsorbed layer will take place in a manner more or less analogous to that analyzed by Volmer (1932, 1938); that the additional amount adsorbed because of the lower temperature will more than compensate for the positive temperature coefficient of surface diffusion; and that the DDT is eluted at the cuticle-cell interface. These assumptions are conceivable, but they make such a formidable list that everyone, the present authors included, will await demonstration of their reality. The other two explanations suggested in point 5 above are purely hypothetical and as such have no support at present.

The most interesting parts of the data presented are the clear demonstration of two opposed temperature coefficients of opposite sign and the localization of the negative component to absorption through the cuticle of the body wall. Under one set of circumstances (high DDT concentration) the results are dominated by the set of reactions involving a positive temperature coefficient; in another set of circumstances (low DDT concentration) the results are dominated by a different reaction or set of reactions involving a negative temperature coefficient. The fact that we are indeed dealing with two different reactions (in contrast to the situations studied by Johnson *et al.* [1942] and Johnson and Lewin [1946]) seems clearly indicated by the differences between internal and external application. Clearly, both sets of reactions must be involved in all immersion tests, one or the other dominating the picture depending on the particular circumstances. A calculation of the Q_{10} for the cuticle penetration or of the Q_{10} for the toxic action was not attempted because several or many reactions might be involved. A computation of these would seem to be meaningless, since certainly we are dealing with a balance of two or

more reactions with coefficients of opposite sign.[4]

It does not seem feasible to attempt comparing our data with the more precise data of Johnson *et al.* (1942, 1946) on temperature effects on drug action in bacteria. It might be appropriate, however, to point out that we did not find a "temperature optimum" for DDT action within the viable range for our animals. The data of Johnson *et al.* do not seem to be complicated by absorption difficulties, whereas we obtain a negative temperature coefficient only from external applications. It is not inconceivable that absolute reaction rates may be involved in the interpretation of cuticle permeability to DDT, but no analysis of our data comparable to their analysis can be made at present.

It seems that it would be of considerable interest to entomologists that some insecticides show this phenomenon of a negative temperature coefficient for external application at low dosages and other insecticides do not (see references cited by Potter and Gillham; we also have other unpublished data on this point). Numerous persons have suggested a role for adsorption in general permeability and also in the action of contact insecticides (e.g., Hoskins, 1932), but such a role has not been directly proved. If the phenomena recorded in the present paper are interpreted in terms of adsorption, as we favor, this would be the first demonstration of the possibly great biological significance of adsorption in cuticle permeability. Be that as it may, it seems to us that one phase of the action of contact insecticides must involve cuticular phenomena of the sort reported in the present paper, but we would like to emphasize that this can be only one facet of the total picture even in those special circumstances where these cuticular phenomena appear to dominate the picture.

SUMMARY

1. (*a*) Following external application, highly susceptible arthropods show either a positive or a negative temperature coefficient for mortality as a function of DDT concentration or dosage. At high concentrations this is positive, at low concentrations negative. (*b*) Following injection, a positive temperature coefficient is always obtained, irrespective of dosage (= concentration). (*c*) DDT can be removed from colloidal suspension by arthropod cuticle and then recovered from the cuticle. (*d*) The results of pretreatments at various temperatures usually follow prediction.

2. The data show that at low concentrations DDT penetrates the arthropod cuticle more effectively at low temperatures. This negative temperature coefficient at low concentrations is shown to be due to properties of the body wall, probably of the cuticle itself. Possible explanations are discussed.

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[4] Any one interested in so doing can readily select figures from the curves presented herein, substitute the values in the Arrhenius equation, and, by simple arithmetic, obtain various μ-values for different conditions. The present authors do not see how such numerical values could have any real significance in the present case.

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DETERMINATION OF REPRODUCTIVE RATES IN RAT POPULATIONS BY EXAMINATION OF CARCASSES[1]

JOHN T. EMLEN, JR.,[2] AND DAVID E. DAVIS

INFORMATION about reproductive rates is fundamental to an understanding of rat populations and their control. This paper presents a method for calculating birth rates from dissections of specimen series.

The practice of staging large-scale trapping and poisoning campaigns against rats often provides extensive series of freshly killed specimens suitable for statistical study. Little use has been made of this material in the past except to record the species composition, the sex ratio, and at times the flea index of the population sample. Data on the incidence of pregnancy have been recorded in a number of instances and have provided some basic information on seasonal and geographic variations in breeding activity (Buxton, 1936).

The value of pregnancy data in obtaining absolute measurements of litter production and birth rates, however, have been only partially realized. Williams (1929) translated pregnancy-incidence data into terms of reproductive rate. The incidence of pregnancy in a population, he contends, is equal to the frequency of occurrence of pregnancy in the average female of that population. In other words, if 25 females are pregnant in a series of 100 collected from a stable population, then the average female in that population is, at that season, pregnant 25 per cent of the time. Since

[1] Contribution from the Rodent Ecology Project of the Department of Parasitology, School of Hygiene and Public Health, Johns Hopkins University. The work reported in this paper was conducted under a grant from the International Health Division of the Rockefeller Foundation.

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we know the length of the gestation period, it is a simple matter to calculate the number of litters that this average female will produce in a given period.

Williams (1929) pointed out that in applying this formula it is necessary to distinguish between visible pregnancy and total pregnancy, since the presence of embryos cannot be detected on gross examination during the first few days of pregnancy. Laboratory studies of tame rats have shown that embryos remain as floating blastocysts in the oviduct until the latter part of the 4th day and become implanted in the uterus on the 6th day (Huber, 1915). Rapid growth of both embryo and uterine mucosa commences at about this time, and the implantation sites become externally visible shortly thereafter. Changes in the uterus progress in an orderly manner during the remainder of the gestation period and may be used to indicate the stage of advancement.

The first objective of the present paper is to present a normal table of the progress of pregnancy based on conspicuous features and gross uterine measurements for practical application to field data.

The second objective is to present formulas for the determination of litter production and birth rate and to apply these formulas to a series of rats taken in connection with control campaigns in Baltimore, Maryland.

MATERIAL AND METHODS FOR PREPARING THE NORMAL TABLE

Laboratory rats of the albino or hooded strains, weighing between 165 and 255 gm., were used in preparing the normal table. Admittedly, it would have been better to have used captive wild animals, but the technical difficulties of handling them were not overcome. Laboratory rats have a gestation period of 21–23 days (Griffith *et al.*, 1942), while wild Brown Rats (belonging to the same species) have a period of $23\frac{1}{2}$–$25\frac{1}{2}$ days according to Miller (1911).

Females for the study, mostly virgins, were placed in groups of ten to fifteen in cages measuring $30\times45\times60$ cm. and were supplied at all times with a surplus of water, dog chow, and occasionally garden greens. Males, four at a time, were placed in the cages with the females and left there for about a week. During this period of exposure to males, each female was carefully examined every morning for the presence of sperm in the vaginal smear. All inseminated females were readily detected by this method; only one so identified failed to become pregnant.

Since insemination occurred during the early hours of night in caged wild rats (Miller, 1911) and in laboratory rats at the Wistar Institute colony (Farris, personal communication), the gestation periods were measured from the evening of the night preceding the finding of sperm in the vagina. Inseminated animals were removed to individual cages or to small group cages and held for varying periods of time as desired. They were usually killed in the morning, and measurements were made of the uterus and the uterine swellings immediately after death. Only the right horn was used. The entire reproductive tract was then placed in 4 per cent formaldehyde for fixation and preservation. Specimens were remeasured and photographed after fixation.

GROSS CHANGES IN UTERUS WITH ADVANCING PREGNANCY

Measurements of the uterus and of uterine swellings (containing embryos) are presented in Table 1. No gross

changes were noticed in the external appearance of the uterus during the first 5 days of pregnancy (Fig. 1, *A*). A vague opaqueness at certain points along the uterine horn were detected on the 6th day ($5\frac{1}{2}$ days after insemination) upon close examination before preservation (Fig. 1, *B*). These marks cannot be measured and cannot be reliably counted; it seems unlikely that they would be noticed at all in ordinary field examinations. Elongate whitish areas, only slightly wider than the tube itself, were seen and readily counted on the 7th-day specimen (Fig. 1, *C*). This can be considered for practical purposes as the first day of visible pregnancy. The swellings increased rapidly in width but not in length during the next few days and by the 9th day appeared as small round nodules about double the width of the uterine horn (Fig. 1, *D*). Growth in length of the swellings paralleled growth in width during the next 8 days, so that the swellings maintained a roughly spherical form and the gravid uterus had a nodular appearance (Fig. 1, *E*). Embryos were palpable through the body wall after about the 11th day, which is the midpoint of gestation. A small discharge of blood in the vagina as described by Donaldson (1924) was detected in the 13th-day specimen, at which time enlargement of the abdomen became apparent on handling the animals. The uterine horn between swellings remained narrow and constricted until about the 18th day, when a pronounced

TABLE 1

NORMAL TABLE OF GROWTH OF UTERUS AND UTERINE SWELLINGS DURING PREGNANCY IN LABORATORY RATS

Stage of Gestation (Days)	No. of Uterine Swellings (Implanted Embryos)	Uterus (in Mm.) (Right Horn)		Uterine Swellings (in Mm.)*	
		Length	Width	Length	Width
0	0	46	4.4	.....	.....
$4\frac{1}{2}$	0	37	3.0	.....	.....
$5\frac{1}{2}$	?	38	3.0	.....	.....
$6\frac{1}{2}$	9	49	2.4	4.0±	2.6(2.4–2.8)
$7\frac{1}{2}$	6	53	2.4	4.2(3.8–4.8)	3.0(2.9–3.0)
$7\frac{1}{2}$	9	41	2.4	3.9(3.6–4.1)	3.7(3.6–3.8)
$8\frac{1}{2}$	8	51	2.0	4.7(4.5–5.2)	4.5(4.3–4.7)
$8\frac{1}{2}$	10	33	2.2	4.0(3.9–4.1)	4.7(4.6–4.8)
$9\frac{1}{2}$	10	37	2.5	4.1(3.9–4.3)	5.2(5.0–5.3)
$9\frac{1}{2}$	2	30	2.4	4.0(3.9–4.0)	5.7(5.3–6.0)
$10\frac{1}{2}$	13	60	2.6	5.5(5.4–5.6)	6.6(6.5–6.6)
$11\frac{1}{2}$	13	40	2.4	5.0(4.2–5.2)	7.8(7.5–8.2)
$12\frac{1}{2}$	12	63	2.0	7.5(6.6–8.5)	8.6(8.0–9.5)
$13\frac{1}{2}$	11	90	2.5	10.3(9.0–11.0)	11.0(10.5–11.5)
$14\frac{1}{2}$	10	72	2.3	11.0(10.5–11.5)	12.0(11.5–12.5)
$15\frac{1}{2}$	6	52	4.0	13.8(13.0–14.0)	14.7(14.0–15.0)
$16\frac{1}{2}$	10	85	5.5	15.0(14.0–16.0)	14.5(14.0–15.0)
$16\frac{1}{2}$	5	74	3.9	16.5(16.0–17.0)	15.5(15.0–16.5)
$17\frac{1}{4}$	7	?	4.8	22.0(21.0–22.5)	17.5(17.0–18.0)
$18\frac{1}{2}$	13	140	10.0	24.0(22.0–25.0)	19.5(18.5–20.0)
$19\frac{1}{2}$	8	215	12.0	25.0(23.0–27.0)	20.5(19.5–21.0)
$19\frac{1}{2}$	10	230	14.0	25.0(24.0–27.0)	20.5(19.5–21.0)
$20\frac{1}{2}$	11	210	14.0	31.5(27.5–34.0)	19.5(18.0–20.0)
$21\frac{1}{2}$	9	180	15.0	35.0(34.0–36.0)	20.5(20.0–22.0)

* The figures represent the average and those in parentheses the minimum and the maximum.

elongation of the swellings occurred, accompanied by an apparent relaxing of the uterine wall so as to change the whole appearance of the horn from a nodular to a tubular form (Fig. 1, *F*). Growth of the swelling after this time was largely linear.

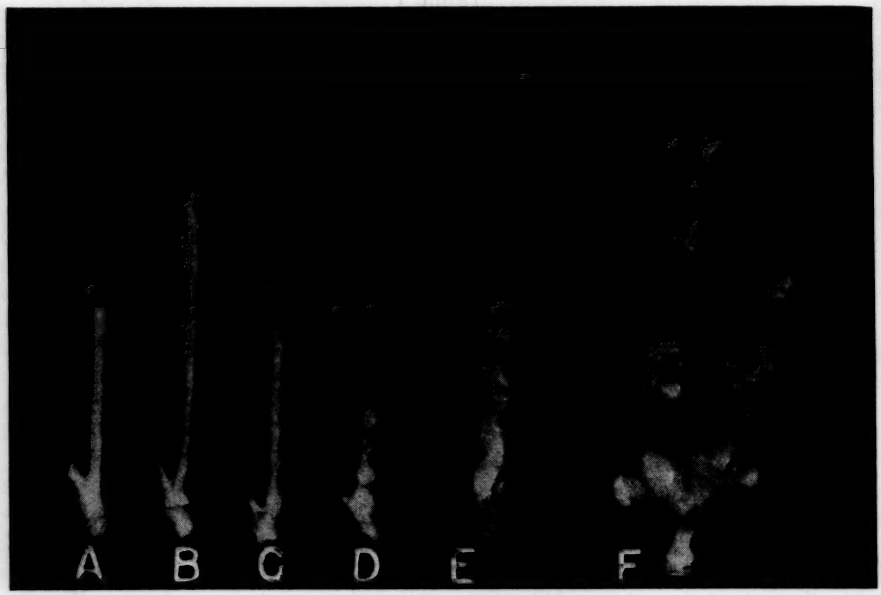

FIG. 1.—*A*, right horn 4½ days after insemination (Phase I). *B*, right horn 5½ days after insemination (Phase I). *C*, right horn 6½ days after insemination (Phase II). *D*, right horn 8½ days after insemination (Phase II). *E*, right horn 16½ days after insemination (Phase III). *F*, right horn 18½ days after insemination (Phase IV).

The principal changes with advancing pregnancy are conveniently divided into 4 phases of roughly equal duration (Fig. 2). The characteristic features of these phases are given in Table 2.

Accepting Miller's figure for gestation in the wild animal and assuming that the longer time is reflected equally in each of the four phases, these become respectively 6, 7, 5½, and 5½ days in duration in wild Brown Rats. It is possible, of course, that the longer time is reflected unequally in the four phases, but evidence to decide this point is lacking.

DETERMINATION OF REPRODUCTIVE RATES

The measurements of uterine swellings here presented indicate that a wild rat is visibly pregnant on gross examination of the uterus during 18 days of each pregnancy. This figure would apply to the occasional prolonged pregnancies associated with concurrent lactation, as well as to the usual 24-day periods, for prolongation has been shown to be due to delayed implantation rather than to a retardation of growth after implantation (Krehbiel, 1941).

With this figure for the duration of visible pregnancy, it is possible to deter-

mine the average frequency of occurrence of pregnancy in females by applying the equation

$$F = I \times \frac{t}{18},$$

where

F = Frequency of pregnancy,
I = Incidence (percentage visibly pregnant),
t = Length of time during which specimens were collected.

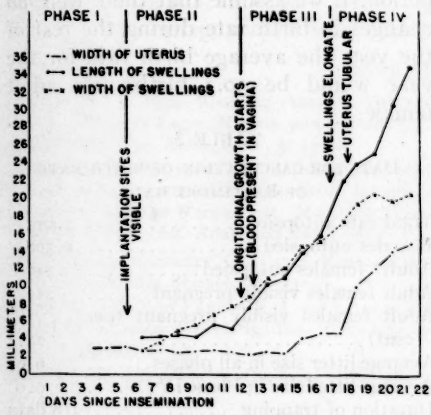

FIG. 2.—Gross uterine measurements related to advancing pregnancy. Width of uterus is the mesometrial-antimesometrial dimension (breadth) near the center of the horn at some distance from an implantation site. Length of uterine swellings is the crown-rump length of an average embryo measured through the uterine wall. Width of uterine swellings in the width of the uterus at its widest point at the site of an implanted embryo.

If it were not for the occasional loss of an entire litter within the reproductive tract after the 6th day (when pregnancies become grossly visible), this figure of pregnancy frequency would be equal to the rate of litter production. No measure of this mortality was made in the present study, and an attempt to compare pregnancy incidence in Phases II and IV was inconclusive because of the lack of sharpness of the period limits. Perry (1945), comparing the number of corpora lutea with the number of embryos in a series of one hundred and thirty-one rats, found only three in which the litters had been lost entirely, and he conjectured that such losses are most likely to occur at the time of implantation. The correction factor needed for translating pregnancy frequency into terms of litter production would, therefore, probably be of the order of 2 or 3 per cent or less.

Birth rate may be determined by multiplying the rate of litter production by the number of young in each litter at parturition. The average number of embryos per pregnant female is inaccurate as a measure of litter size at parturition because of intra-uterine mortality of embryos, resulting in an appreciable reduction in litter size between the time of implantation and parturition. Perry (1945) calculated the total loss of ova and embryos at 20.5 per cent, based on corpora lutea counts. Apparently, very little loss occurs during the last 5 days of pregnancy, so an average of embryo counts in the last quarter of pregnancy (Phase IV) should approximate litter size at parturition and provide a practical unit for application to series of specimens. Another method of obtaining the desired figure would be to establish a general correction factor based on large series of specimens and apply this value to other small series.

Birth-rate figures as well as other types of data obtained from field series are subject to error arising from variations in the

TABLE 2

RELATION OF UTERINE CHARACTERISTICS TO DAYS SINCE IMPREGNATION

Phase	Length of Swellings (Mm.)	Width of Uterus (Mm.)	Duration (Days)	Figure
I.....	Not visible	<3.0	5½	1, *A*; 1, *B*
II.....	<5.5	<3.0 (nodular)	6½	1, *C*; 1, *D*
III...	6.0–17.0	3.0–6.0 (nodular)	5	1, *E*
IV	>17.0	>6.0 (tubular)	5	1, *F*

technique of population sampling. Practically nothing is known about the relative responsiveness of pregnant and non-pregnant rats to traps and poison sets. Employment of large samples is, perhaps, the best assurance against errors from this source. Complete records of sex and age groups should be kept for any series, and, as Buxton (1936) has pointed out, only adult females should be included in the pregnancy computations.

For practical purposes a good criterion for differentiating adult from immature female rats is perforation of the vagina. Immature female rats have the vagina closed, but in adult females it is open at all times. All females in the adult category or an arbitrary weight class of heavy animals, well above the maximum immature weight, may be used for the pregnancy computations.

REPRODUCTIVE RATE IN A BALTIMORE RAT POPULATION

Autopsies were performed on 3,015 Brown Rats taken at random from a series of rats trapped between August 18, 1943, and March 23, 1944, from a seventy-block area in a downtown residential section of Baltimore, Maryland. The autopsy procedure included observations and measurements of size, sex, occurrence of pregnancy, condition of uterus, number of embryos, and size of the uterine swellings. Data pertinent to the present problem are given in Table 3.

Applying the equation for determination of pregnancy frequency,

$$F = 0.284 \times \frac{218}{18},$$

we obtain

$$F = 3.44 .$$

Correcting this figure for an estimated 3 per cent intra-uterine loss of entire litters, we conclude that the average adult female brought 3.34 litters successfully to term in the 218 days of the study period. This is a rate of 1 litter every 65 days and is very close to the figure of 1 litter every 2 months proposed by Lantz (1917) for other rat populations.

Using the average embryo count during Phase IV of gestation as the litter size at parturition, we obtain a birth-rate figure of $3.34 \times 9.0 = 30.0$ young per average adult female during the 218-day period. If we assume that there were no changes in birth rate during the rest of the year the average birth rate for the year would be 50.3 births per adult female.

TABLE 3

DATA FOR CALCULATION OF BIRTH RATE OF BALTIMORE RATS

Total rats autopsied	3,015
Females autopsied	1,568
Adult* females autopsied	746
Adult females visibly pregnant	212
Adult females visibly pregnant (per cent)	28.4
Average litter size in all phases	9.9
Average litter size in Phase IV	9.0
Duration of trapping	218 days

* Females over 250 gm. in weight.

Eaton and Stirrett (1928) used another method to calculate birth rates. They assumed that pregnancy was grossly visible for 16 days and that half the females are adult. If the rate is 1 litter per year, then the chances are 1 in 45 ($16/365 \times \frac{1}{2}$) of finding a litter, i.e., 2 per cent of all females are pregnant. Since 7 per cent of the female rats examined by these authors in New Bedford, Massachusetts, were visibly pregnant, there are 3–4 litters per year per female. Using the figure of 13.5 per cent of all females pregnant in Baltimore, we obtain the reasonable figure of about 7 litters per year.

SUMMARY

To determine the gross characteristics of successive stages of pregnancy of Brown Rats, a normal table of the prog-

ress of pregnancy was prepared from a series of albino rats in the laboratory. Pregnancy was divided into four phases (illustrated) which have in the wild Brown Rat durations of 6, 7, $5\frac{1}{2}$, and $5\frac{1}{2}$ days. Since the embryos are not visible during the first phase, a rat is visibly pregnant at autopsy for only 18 days. To determine the average frequency of pregnancy in a series of wild rats, the formula $F = I \times t/18$ is used, where F is frequency of pregnancy, I is incidence (per cent visibly pregnant), and t is the length of time during which specimens were collected for the series. Birth rate equals F times average young per litter, corrected for intra-uterine mortality. In Baltimore, autopsies of 746 adult (over 250 gm.) wild females showed 28.4 per cent visibly pregnant and an average litter size of 9. Hence an average adult female had one litter every 65 days and produced 30 young during the 218-day sampling period.

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A SEXUAL TRANSFORMATION OF THE OSSEUS BULLA IN DUCK EMBRYOS FOLLOWING ADMINISTRATION OF ESTROGEN[1]

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STUDIES on the effect of estrogens upon the embryonic reproductive system of the male chick have shown that complete sex inversion may occur when appropriate doses are administered. In order to be certain of the genetic sex of chick embryos treated in this manner, some investigators have relied on sex-linked plumage characters (Willier, Gallagher, and Koch, 1937). In similar work on pure-bred White Pekin ducks (Lewis, 1946), in the absence of a sex-linked plumage character, some other constant feature as an indicator of the genetic sex of treated individuals was sought. The osseus bulla, a male sex character, which is frequently used in sexing duck embryos (Kortright, 1942), was considered a structure which might serve this purpose.

[1] This investigation was aided by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago. Grateful acknowledgment is made to Dr. Erwin Schwenk, of the Schering Corporation, for the alpha-estradiol benzoate (Progynon-B); to Dr. J. A. Morrell, of Squibb and Sons, for the diethylstilbestrol; and to W. F. Straub and Company, for the hexestrol used in these experiments.

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The characteristics of the osseus bulla, which is located on the trachea of the male, as well as those of the female trachea, which lacks this apparatus, were described by Zawadowsky (1928). It is located at the point of bifurcation of the trachea to form the bronchi and is a part of the so-called "inferior larynx," which is the organ of voice production. The

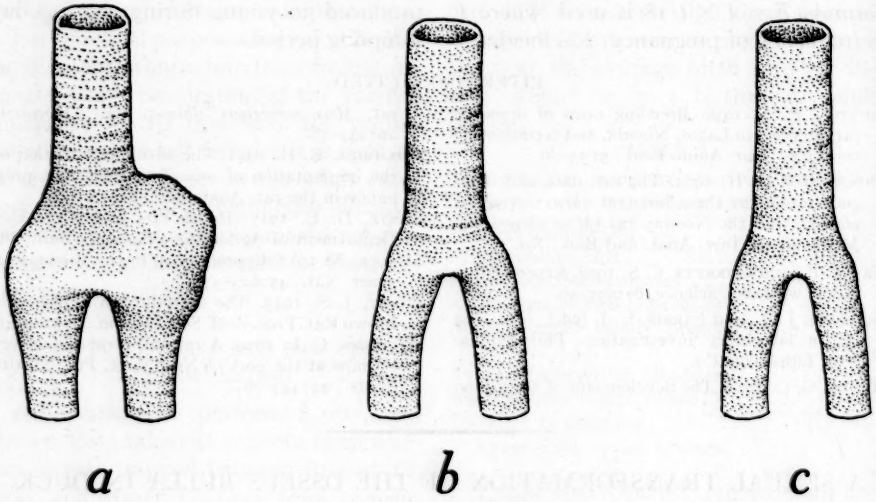

FIG. 1.—(*a*) Ventral view of a portion of the trachea of a normal male embryo, 24 days incubation, showing the osseus bulla. (*b*) Ventral view of a portion of the trachea of a male embryo treated on the 4th day with 2.50 mg. of hexestrol and killed on the 25th day of incubation. Note feminization of osseus bulla as compared with Fig. 1, *a*. (*c*) Ventral view of a portion of the trachea of a normal female embryo, 24 days incubation.

osseus bulla in the male is asymmetrical, growing out laterally and anteriorly on the side of the left bronchus (Fig. 1, *a*). This accounts for the fact that the lower larynx of males is larger than that of females. In females the trachea bifurcates into the two more or less symmetrical bronchi (Fig. 1, *c*).

MATERIALS AND METHODS

The White Pekin duck was used exclusively throughout these experiments. Fertile eggs were obtained from a hatchery in Ohio and a farm in Indiana[3] and were incubated in electrically heated incubators in the laboratory. For details concerning incubation and injection procedures the reader is referred to the study by Lewis (1946).

The hormones employed in the study on the effects of estrogens upon the embryonic reproductive system were alpha-estradiol benzoate, diethylstilbestrol, and hexestrol (Lewis, 1946). Unfortunately, treatment with the first two hormones named had been concluded and most of the tracheae discarded before our attention was directed to the osseus bulla as a possible indicator of the genetic sex of treated embryos; consequently, only the effects of hexestrol upon this character are described at this time.

[3] Twentieth Century Hatchery, New Washington, Ohio, and Mrs. Blanche Tormohlen, Portland, Indiana.

RESULTS

When embryos treated with 2.50 mg. of hexestrol on the 4th day of incubation and killed on the 25th day were examined macroscopically, the tracheae showed no sexual dimorphism whatsoever (Fig. 1, *b*). The question therefore arose as to whether the White Pekin was one of those species of ducks in which the tracheae of males do not possess the osseus bulla (Miller, 1926). However, examination of the tracheae of controls soon showed that this was not the case, for they clearly displayed sexual dimorphism.

Embryos injected with 2.50 mg. of hexestrol on the 6th, 8th, 9th, or 10th day and killed between the 19th and 28th days of incubation displayed tracheae clearly of the female type, and the sexes could not be distinguished by examination of the windpipe alone, as was the case in controls (Table 1). However, when injections were made on the 12th day of incubation, males could be separated from females when examined on and after the 22d day, though the osseus bulla was not typically male. It bulged slightly to the left but was considerably reduced in size as compared with controls. Its condition in such cases may be described as "intersexual." In the case of male embryos treated on the 14th day, the osseus bulla was larger and more male-like when examined between the 25th day of incubation and the 4th day after hatching, though not entirely normal, while in those injected on the 16th day and examined between the 28th day of incubation and the 2d day post-hatch it exhibited only slight modifications. When injections took place as late as the 18th day, the hormone was found to have little or no effect upon the trachea when examined just before or after hatching (Table 1), showing that the form of the osseus bulla of the White Pekin duck apparently can no longer be modified by

TABLE 1

SUMMARY OF THE EFFECTS OF HEXESTROL

Injection Age*	Quantity Injected (Mg.)	No. of Eggs Injected	Embryos Killed			Character of Osseus Bulla
			Age*	Male†	Female	
4	2.50	145‡	25	6	2	All female
6		45	19–25	9	5	All female
8		30	19–25	8	6	All female
9		25	23–27	8	3	All female
10		29	25–28	4	1	All female
12		53	22–2§	12	13	Intersexual and female
14		62	25–4§	11	7	Intersexual and female
16		30	28–2§	5	3	Intersexual and female
18		16	27–4§	2	2	No effect
4 and 6	3.75	60	All dead by 10th day			
Totals				52	37	
Sex ratio				58	42	

* Incubation days.

† Sex determined by histological study of gonads.

‡ The discrepancy between number of eggs injected and embryos killed in all cases in the table is due to the death of embryos in the interim.

§ Post-hatching.

estrogen administration at this stage of embryonic development.

The time of irreversible determination of the osseus bulla, therefore, coincides fairly well with the period at which estrogens were found to be no longer capable of modifying the persistence of the right Müllerian duct in female embryos and the time when complete degeneration of these ducts in males has been attained (Lewis, 1946).

DISCUSSION

The osseus bulla is a male sexual character located on the trachea in certain species of ducks. The fact that this character may be feminized under the influence of estrogen raises certain interesting questions, the chief of which concerns its method of determination in development. Whether its development is normally influenced by sex hormones, as has been shown to be the situation in the case of secondary sexual characters in the domestic fowl (Domm, 1927, 1929; and others), has not been definitely established. It is conceivable that male-female genetic differences are solely responsible for the sexual dimorphism of the trachea and that at an early period in its development estrogens are experimentally capable of exercising an inhibitory effect by overriding the male genetic influence and suppressing formation of the osseus bulla. On the other hand, if sex hormones do normally operate in the development of this structure, then estrogen predominating in the female might prevent its appearance, or a preponderance of androgen in the male might exert a stimulating effect. If the latter theory is true in the White Pekin duck, then castration of males should prevent development of the osseus bulla in that sex, or the administration of androgens in females should stimulate its appearance. Such data are not as yet available.

It should be pointed out that earlier experiments have demonstrated that sex hormones do not become evident prior to hatching in chicks (Domm and Dennis, 1937; Domm, 1937), despite the fact that embryonic combs (Domm, 1937) and gonaducts (Willier, Gallagher, and Koch, 1937) are capable of responding to androgen and estrogen, as indicated by injections into the incubating egg. There is no evidence at present that would lead us to suspect that the situation in the White Pekin duck, in which the embryonic trachea of zygotically determined males has been found to be responsive to estrogen, is any different from that observed in the chick with respect to the role of sex hormones in embryonic development. We know of no other character in birds, displaying sexual dimorphism at the time of hatching, which has manifested a similar transformation following the administration of sex hormones. It is not unlikely that the cloacal papillae in the domestic fowl, used in sexing newly hatched chicks, or the copulatory organ in the duck, both becoming sexually dimorphic during the period of embryonic life, might respond in a similar manner.

Zawadowsky (1928) performed castration experiments on both sexes in domestic and wild Mallard ducks ranging in age from two to six weeks. No change was observed in the voice or vocal apparatus of either sex, and he therefore assumed that this character, developing differently in male and female, develops independently of sex hormones. However, he felt that it was possible that results might be different with earlier castration. Unfortunately, this problem has not been further investigated.

SUMMARY AND CONCLUSIONS

The results obtained in this study show grossly that the osseus bulla of the

White Pekin duck male undergoes a transformation in the female direction following injection of the estrogen, hexestrol, into the incubating egg between the 4th and 10th days of incubation.

When injections were made between twelve and sixteen days, males could be distinguished from females, though the osseus bulla was not typically male but rather intersexual in character. Injections on or after the 18th day had no noticeable effect upon this character.

The osseus bulla therefore cannot be used as a reliable criterion for distinguishing the sex of White Pekin duck embryos following injections of hexestrol during early incubation.

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THE KILLING ACTION AND RATE OF PRODUCTION OF SINGLE PARTICLES OF PARAMECIN 51[1]

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I. INTRODUCTION

IN HIS studies on *Paramecium aurelia*, Sonneborn (1938, 1939, 1943, 1947*a*, *b*) has found that certain stocks ("killers") liberate into the culture fluid in which they live a substance called "paramecin," which kills certain other stocks ("sensitives") of the same species as well as of other species of *Paramecium*. In studies made eight years ago, Sonneborn (personal communication) observed that in a mixture of known numbers of killer and sensitive paramecia, the number of sensitives killed during a given period varied with the number of killer animals present. The relations were such as to suggest, further, that each killer animal produced so little paramecin in the course of a day that it could kill very few sensitives. At his suggestion, there-

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fore, a systematic study was made in order to follow up these clues.

In the present study the killer stock employed was Sonneborn's (1943, 1945*a*, *b*) stock 51 of variety 4; and the standard sensitive stock was his stock 31 of variety 8 (Sonneborn and Dippell, 1946*a*, *b*).

II. METHODS

Sonneborn (1939) discovered that sensitive paramecia are killed regardless of whether they are placed directly with the killers or in fluid from which the killers have been removed. The general procedure followed in the present experiments was to observe the action of a known volume of fluid (usually 0.1–0.2 cc.) in which a known small number of killer animals had lived for 5 hours. Under the conditions employed, this 5-hour period represents the time that ordinarily elapses between two successive divisions. The killer animals were introduced into the fluid just after the completion of one fission and were removed from it just before the next. After the killer animals had been removed, a certain number of the sensitives in a constant volume of fluid was introduced into this fluid. During the 5-hour period when the killers were in the fluid, as well as after they had been removed and the sensitives added, the tests were kept in a constant-temperature box at 26.5°–27° C. On each of the 3 days following the initiation of an experiment, the sensitive animals were examined. All dying animals or corpses were removed with a micropipette, and counts were made of the numbers so removed.

This procedure was used in two main ways. In the first the number of sensitive animals killed was determined when the number of killers was varied, while the number of sensitive animals used was approximately the same (from 100 to 200). In the second the same varied series of killers was employed as in the first, but now the number of sensitives used was also varied at each level in the killer series. These two methods thus provide means of measuring separately the effects of varying the amount of paramecin and the number of sensitive animals. In each case the effect is measured by the number of sensitive animals visibly acted upon by paramecin.

Paramecin produced by stock 51 produces visible effects on sensitives that are readily recognized (Sonneborn, 1943). A characteristic bulge gradually forms on the aboral side. This is accompanied by a marked slowing of all activities, so that such an affected animal is very easy to detect among the rapidly swimming normal animals Not even sharp tapping of the depression slide or forcing of the animal in and out of a pipette will stimulate it to any swifter movement. Eventually, it rounds up and sinks to the bottom of the dish, a corpse that may take 18 or 24 hours or even longer to disintegrate. Altogether, the response of sensitive animals is so striking that, if only one animal is affected among a group of several hundred in 0.1 or 0.2 cc., this one can be spotted even with magnifications as low as 15 diameters. Quantitative studies of the sort here reported are thus made possible.

The fluid used in setting up these tests was, in the earlier experiments, a standard baked lettuce infusion (Sonneborn, 1936) into which the bacterium *Aerobacter aerogenes* had been inoculated one or two days earlier (Sonneborn and Dippell, 1943). This was the same culture medium as that used for the cultivation of the paramecia. In later experiments, after Sonneborn, Jacobson, and Dippell (1946) had discovered that the number of sensitives killed is reduced when abundant food is available, the tests were set up in supernatant fluid from centrifuged cultures that had nearly exhausted the food supply. In order to reduce the avail-

able food still more, especially in experiments in which small numbers of sensitive animals were employed, this fluid was diluted 1:1 with 0.01 M NaCl. The difference in the results following this change in technique did not seem radical: the maximum counts of lethally affected sensitives which were obtained with the food medium were obtained a little more frequently with the supernatant fluid.

The killer and the sensitive animals used in setting up the tests were taken from test-tube cultures. In the later experiments the animals were grown at known rates by control of the food supply according to the method of Preer (1946 and personal communication). The animals were grown at different rates for different experiments, the killers at one, two, or three fissions per day, the sensitives at two or three fissions per day. In order to avoid carrying over paramecin from the stock killer cultures, the animals to be used for experiments were washed by carrying them through five transfers in slide depressions or through three transfers in Syracuse dishes. The sensitive animals were also washed at least once.

As a control for each set of experimental tests, animals of the sensitive stock 31 were introduced into fluid for 5 hours, after which they were removed and replaced by 100–200 other sensitives. Two more sets of sensitive animals were also kept under observation in each experiment, to make sure that deaths from other causes were not occurring.

III. EXPERIMENTAL RESULTS

A. THE NUMBER OF SENSITIVE ANIMALS KILLED WHEN THE AMOUNT OF PARAMECIN IS VARIED AND THE NUMBER OF EXPOSED SENSITIVES IS KEPT CONSTANT

1. *Results of a preliminary group of experiments in which 1, 2, 4, 8, 16, and 32 killers were introduced into fluid with the sensitives and allowed to remain.*—A preliminary group of four experiments was carried out in a somewhat different way from that just described as the general method used in all other experiments. In these first experiments the killers were not removed from the fluid before the sensitives were added but, instead, were introduced into the same fluid with them.

TABLE 1

THE EFFECT OF INTRODUCING A VARIABLE NUMBER OF KILLERS INTO THE SAME FLUID WITH THE SENSITIVE ANIMALS OF THREE DIFFERENT STOCKS, 31, 32, AND 29, AND ALLOWING THEM TO REMAIN

At the end of 24 hours the lethally affected individuals were counted. The results in the table are the averages of four experiments.

No. of Killers	No. of Sensitive Animals Killed in 24 Hours		
	Stock 31	Stock 32	Stock 29
1	16.0	12.2	6.7
2	26.2	18.7	15.5
4	58.7	42.7	32.7
8	106.7	84.2	53.5
16	175.0	176.7	102.0
32	448.3	351.2	197.5
Total killed	830.9	685.7	407.9
No. of sensitives killed per original killer: mean of means	13.6	10.8	6.9
Ratio	1.9 :	1.5 :	1.0

Into about 0.5 cc. of food medium containing 150 sensitives in two experiments and 400 in two others, 1, 2, 4, 8, 16, and 32 killers were introduced and allowed to remain. At the end of 24 hours the counts of lethally affected individuals were made by removing them with a capillary pipette. Table 1 gives the averaged results of these four experiments. The number of sensitives killed represents the effects of the original killers and of their progeny during the 24 hours. Since no attempt was made to select small postfission killers, as was done in all later experiments, there was considerable

variation in the time at which the first fission occurred and therefore differences in the total number of killers produced in the 24 hours from each killer that had been introduced at the beginning of the experiment. When allowance is made for a certain amount of variation in the production of paramecin as a result of such variation in the number of progeny produced from each killer, one is struck by the fact that there is an approximate doubling of the number of sensitives killed as the number of killers is doubled.

In tests set up at the same time as those with the standard sensitive stock 31, two other sensitive stocks were used: stocks 29 and 32 of variety 4 (Sonneborn and Dippell, 1943). With these stocks, also, there is an approximate doubling of the number killed as the number of killers is doubled, but this doubling is at a lower level than it is with stock 31. For any "dosage" of paramecin in stock 29, only about half as many animals are killed as in stock 31, while stock 32 gives intermediate results. From these four experiments, since only the 24-hour count of affected individuals was made, it would be impossible to tell whether the differences are to be interpreted as the result of a slower response to paramecin of stocks 29 and 32; of differences in the proportion of sensitive animals in any given number; or of some other unknown factor entering into the situation. Such differences in sensitivity to paramecin among different stocks of paramecia have also been found by Sonneborn (1938 and personal communication). It is planned to make a thorough investigation at a later time of the interesting question of the causes of the differences. Here no more can be said than that at the end of 24 hours, with the killers left in with the sensitives, there is the same approximate doubling of the number killed, as the number of killers is doubled, in stocks 29 and 32 as in stock 31, though the absolute values are different in the three cases. The finding of a fairly constant relationship between the number of killers and the number killed in these four preliminary experiments led to the further study of this relationship under the more carefully controlled conditions already described in Section II.

2. *Results when 1, 2, 4, 8, 16, and 32 killers were introduced into fluid for 5 hours, then removed before the sensitives were added.*—As already mentioned, the amount of paramecin in a given volume of fluid may be varied by varying the number of killer animals that have lived in it for the standard interval of 5 hours. Forty experiments were conducted with this technique. Usually in each experiment, 1, 2, 4, 8, 16, and 32 killer animals were used, though in some experiments one or two members of this series were omitted. After these diverse numbers of killers had remained in 0.1–0.2 cc. of fluid for 5 hours, they were removed with a capillary pipette, and the sensitive animals were then introduced into the fluid in which the killers had liberated paramecin. In 6 of the experiments, from 250 to 600 sensitive animals were used; in the other 34, from 100 to 200 sensitives were used.

The results of these 40 experiments, together with the mean number of sensitive animals killed for each killer or group of killers originally introduced into the fluid, are shown in Table 2. Ninety-two other tests with single killers have been made, but adding the results of these 92 tests to those of the 40 experiments in Table 2 leaves the mean number killed (0.9) unchanged. The combined results of the 132 tests with single killers are: 30 tests with none killed, 85 with one killed, 17 with two killed.

There are several points of interest to be noted in Table 2: (1) The maximum

number of sensitives killed rises with an increase in the number of killers used. Through the first four groups (with 1, 2, 4, and 8 killers) this is a regular rise, the maximum number in each case being equal to twice the number of killers used. (2) There is exact correspondence between the number of killers and the number killed in 29 per cent of the total of 211 tests. (In 5 of the 40 experiments there was very close correspondence in all six members of the series simultaneously. In one of these, 1, 2, 5, 8, 15, and 31 were killed; in another, 1, 2, 2, 9, 16, and 33 were killed.) In the first four groups of tests, there is exact correspondence in 44 per cent of the tests. In 80 per cent of all the tests the number killed is equal to, or less than, the number of killers; in 20 per cent the number killed is greater than the number of killers. The study of individuals that were isolated at the first signs of having been affected by paramecin has shown that occasionally such an individual may undergo an abnormal division and thus form two corpses. This is

TABLE 2

NUMBER OF SENSITIVES KILLED BY FLUID IN WHICH VARYING NUMBERS OF KILLERS HAVE LIVED FOR 5 HOURS

No. of Sensitives Killed	No. of Killers in Fluid for 5 Hours					
	1	2	4	8	16	32
0	8	0	3	1	0	0
1	28	9	1	0	2	0
2	4	14	6	1	1	0
3	.....	3	7	3	0	1
4	.....	3	11	0	2	1
5	.....	.....	4	1	1	0
6	.....	.....	3	6	1	0
7	.....	.....	1	2	1	0
8	.....	.....	1	7	2	0
9	.....	.....	.....	3	1	2
10	.....	.....	.....	2	3	2
11	.....	.....	.....	0	1	0
12	.....	.....	.....	1	4	0
13	.....	.....	.....	0	2	3
14	.....	.....	.....	2	4	2
15	.....	.....	.....	0	1	1
16	.....	.....	.....	1	2	1
17	.....	.....	.....	.....	4	1
18	.....	.....	.....	.....	0	0
19	.....	.....	.....	.....	3	3
20	.....	.....	.....	.....	2	5
21	.....	.....	.....	.....	0	1
22	.....	.....	.....	.....	0	0
23	.....	.....	.....	.....	0	1
24	.....	.....	.....	.....	2	1
25	.....	.....	.....	.....	.....	1
26	.....	.....	.....	.....	.....	1
27	.....	.....	.....	.....	.....	2
28	.....	.....	.....	.....	.....	1
29	.....	.....	.....	.....	.....	0
30	.....	.....	.....	.....	.....	1
31	.....	.....	.....	.....	.....	2
32	.....	.....	.....	.....	.....	0
33	.....	.....	.....	.....	.....	3
Mean no. killed ± standard error	0.9±0.08	2.0±0.1	3.6±0.3	7.5±0.6	12.1±0.9	19.7±1.3

undoubtedly the explanation for some of the cases in which the number killed is greater than the number of killers, but not for all of them. Other evidence which will be presented later makes it clear that under the conditions of these experiments one killer may sometimes produce enough paramecin in 5 hours to affect more than one of the sensitives directly exposed to it. (3) A third point to be noted in Table 2 is that the average number killed rises with an increase in the number of killers. There is close numerical agreement, through the group of eight killers, between the average number killed and the number of killers used. In other words, in these first four groups, as the number of killers is doubled, the average number of sensitives killed is doubled, with the ratios rather close to a 1:1 relationship—1:0.9, 1:1, 1:0.9, and 1:0.9. The discrepancy between the mean number of sensitives killed and the number of killers that had previously been in the fluid is not greater than 10 per cent when 1–8 killers were employed. But it rises to 25 per cent when 16 killers were in the fluid and to nearly 40 per cent when 32 killers were used. The ratios between the number of killers and the average number killed are 1:0.75 with 16 killers and 1:0.6 with 32 killers. Clearly, these two groups require further consideration in order to be understood.

TABLE 3

PROOF THAT PARAMECIN IS REMOVED WHEN 32 KILLERS ARE WITHDRAWN FROM SMALL VOLUMES (0.1 CC.) OF FLUID IN WHICH THEY HAVE LIVED FOR 5 HOURS

Fluid A is the original volume of fluid from which the killers were removed after 5 hours. Fluid B is a second volume (also 0.1 cc.) of fluid through which these killers were quickly washed immediately after removal from fluid A.

Experiment	No. of Sensitive Animals Killed by:		
	Fluid A	Fluid B	Total A+B
1	23	7	30
2	28	4	32
3	22	8	30
Mean no. killed	24.3	6.3	30.6

3. *Analysis of the cause of the disproportionately small effect of larger numbers of killers.*—When one recalls the technique employed in the experiments, a possible explanation of the larger discrepancies in the results with 16 and 32 killers appears at once. The killer animals were introduced into 0.1–0.2 cc. of fluid and were removed with a micropipette at the end of 5 hours. Naturally, fluid is removed with every animal that is removed. The greater the number of animals taken out, the greater is the amount of fluid taken out; consequently, the greater would be the chances of carrying away in the fluid some of the paramecin. Two methods of testing this possibility were devised.

The first method consisted in transferring the killers at the end of the 5-hour period from a drop of fluid of the volume often employed (0.1 cc.) into a second drop of fluid of the same volume and in washing out the pipette in this fluid. The killers were allowed to swim across or around the second drop once and were then immediately removed, so that no killer was in the wash fluid for more than 2–3 minutes. Sensitives were then added to both tests. As Table 3 shows, the number of those killed in the first 0.1 cc. of fluid was considerably lower than the number of killers, with a mean for the three experiments of 24.3; but when those killed in the wash fluid are added to the number killed in the first fluid, the number killed closely approximates the number of killers, with a mean of 30.6 and a ratio between killers and killed of 1:0.9.

The second method of testing the possibility that some of the paramecin may be carried away in the fluid when as many as 32 killers are removed consisted in varying the volumes of fluid (0.05, 0.1, 0.15, and 0.45 cc.) into which 32 killers were introduced, on the hypothesis that the larger the volume of fluid, the smaller would be the chance of picking up some of the liberated paramecin along with the killer animals. After the killers had been removed, the volumes were equalized by bringing them all up to 0.45 cc., and 500–600 sensitive animals were added to each test. The reason for selecting these particular volumes to test was that 0.1 cc. and 0.15 cc. had frequently been used in setting up the tests reported in Table 2; and for comparison with these there would be one volume (0.05 cc.) smaller than had ever been used, as well as one volume (0.45 cc.) very much larger than had ever been used. Standing in the ratio of 1:2:3:9, these volumes should show up a difference if one existed. As is shown in Table 4, the mean number of sensitives killed increased progressively as the volume of fluid increased, until there was a close agreement between the number of killers and the number killed when a volume of 0.45 cc. was employed. There was considerable variation in the numbers killed from test to test, but in any one test there was usually an increase between 0.05 and 0.1 cc. and between 0.1 and 0.15 cc., and always an increase between 0.15 and 0.45 cc. Furthermore, even though the series of tests is short, both the mean and the limits of variation in the tests with 0.45 cc. are now more nearly what one would expect them to be with 32 killers, from the results with 1, 2, 4, and 8 killers, while those in the tests with 0.1 cc. and 0.15 cc. are in agreement with the observations recorded in Table 2.

The rate of increase in the number killed, however, is so great that it is impossible to account for it entirely on the basis of the proportionate volume of fluid removed. From the tests with 0.05 cc., about 74 per cent of the paramecin, on the average, was removed. If an average of 74 per cent of the fluid had been removed in the capillary pipette, about 0.037 cc. would have been taken out with the killers. From the tests with 0.1 cc., an average of 50 per cent of the paramecin was lost, and a corresponding loss of fluid would have amounted to 0.05 cc. From the test with 0.15 cc., about 33 per cent of the paramecin was lost, and this, too, would have meant the loss of about 0.05 cc. of fluid. These figures are too large. It is estimated that the fine micropipette that was being used was not removing more than 0.01–0.02 cc. at the outside limit, a volume which it had first added to the volumes of the tests when the killers were introduced. An additional explanation had therefore to be sought for this increase in killing with the increase in volume.

TABLE 4

KILLING ACTION OF VARYING VOLUMES OF FLUID INTO WHICH A CONSTANT NUMBER (32) OF KILLERS WAS INTRODUCED AND ALLOWED TO REMAIN FOR 5 HOURS

After killers were removed, all volumes were brought up to 0.45 cc.

Experiment	No. of Sensitives Killed in Volumes of:			
	0.05 cc.	0.1 cc.	0.15 cc.	0.45 cc.
1	6	21	35	60
2	7	33	49	52
3	9	19	22	36
4	10	6	11	19
5	14	18	15	21
6	8	13	16	29
7	No test	10	14	24
Mean no. of sensitives killed by 32 killers	9	17	23	34

Superficially, this increase in killing with an increase in volume might seem to be in agreement with the findings of Drzewina and Bohn (1921), Fowler (1931), and others who have studied the phenomena of "group protection" in various animals (for a bibliography of this subject see Allee, 1931). But a closer examination of the conditions of this experiment shows that it is the volume in which the killers spent the 5-hour period and not the final volume that made the difference; for in the first three tests the amount of killing was still small, although the final volume in all was the same. Another reason why this case bears no relation to those cited by the foregoing authors is that in their experiments the number of animals exposed to a toxic substance was kept the same while the volume was increased. Here the number of sensitive animals exposed to the paramecin was increased as the volume was increased, so as to have it proportionately what it was in the other experiments (about 100 per 0.1 cc.).

The hypothesis next considered was that the paramecin was closer to the animals in the smaller volumes than in the larger and therefore had a greater chance of being removed with the animals. The volume relations would, of course, make this true to some extent; but if, in addition, the paramecin tended to collect on the bottom of the dish, then, in general, the animals in the shallower fluids would certainly be closer to the paramecin than those in the deeper fluids, through which the animals swim freely, often near the surface. With the smaller volumes of fluid spread out thinly over the bottom of the slide depression, it would be difficult not to draw up the paramecin with the animals. Tests of this possibility showed that such is actually the case. The results are reported in the next section. Here it is sufficient to bring out the fact that there is an increase of killing with 32 killers when the volume of fluid in which the killers are left is increased, as it was suspected that there would be.

The results of these two methods of analysis demonstrate that, when precautions are taken to minimize the chances of removing paramecin with the killer animals or to recover in a second volume of fluid paramecin lost from the first, the numerical correspondence between the number of killers and the mean numbers of those killed is very nearly perfect, even when larger numbers of killers are employed. Therefore, the conclusion seems justified that, over a period of 5 hours, each killer is, on the average, responsible for the presence in the medium of enough paramecin to kill one sensitive animal. Furthermore, it is now possible to apply to the cases with the larger numbers of killers, as well as to those with the smaller numbers, the even more important generalization that when approximately equal and large numbers of sensitive animals are exposed to varying amounts of paramecin as measured by the numbers of killers, the mean numbers of sensitives killed is doubled each time that the number of killers (and therefore the amount of paramecin) is doubled.

4. *Evidence that paramecin tends to collect on the bottom of the culture dish.*—To test the possibility (mentioned in the preceding section) that the paramecin tends to collect on the bottom of the culture dish, 50 killers were introduced into 1 cc. of fluid in five different tests: A_1, A_2, B_1, B_2, and C. At the end of 5 hours they were removed. The fluid in test A_1 was then thoroughly mixed by forcing it in and out of a pipette. After this mixing process, 0.8 cc. was drawn off and distributed into four other depressions with 0.2 cc. in each, leaving 0.2 cc. in the original depression. Sensitive animals

were then added to each of these five tests. The fluid in test A_2 was treated similarly, except that the mixing process was omitted and the 0.8 cc. was withdrawn with as little disturbance as possible and with the tip of the pipette kept just under the surface of the fluid. Tests into which it was subdivided; but when the top of the fluid was carefully withdrawn without mixing, 70 per cent of the paramecin was left in the original depression when one-half of the fluid was removed, and 50 per cent of it was left when as much as four-fifths of the fluid

TABLE 5

TEST OF THE POSSIBILITY THAT PARAMECIN TENDS TO COLLECT ON THE BOTTOM OF THE CULTURE DISH: COMPARISON OF THE KILLING ACTION OF SAMPLES OF PARAMECIN FLUID REMOVED AFTER THOROUGH MIXTURE AND WITHOUT SUCH MIXTURE

In each test 50 killers had lived in 1 cc. of fluid for 5 hours. Fluids in tests A_1 and B_1 were thoroughly mixed. From A_1, 0.8 cc. was withdrawn and subdivided into four other 0.2-cc. samples. From B_1, half was withdrawn into one other test. From A_2, 0.8 cc. was withdrawn with a minimum of disturbance and distributed into four tests. From B_2, half was similarly withdrawn into one other test. The fluid in test C was left undivided. The results represent averages of two similar experiments.

Test	No. of Sensitive Animals Killed					
	1 (Original Dish)	2	3	4	5	Total Killed
	0.2 Cc.					
A_1 (fluid mixed before removal of 0.8 cc.)	10	9	10	11	11	51
A_2 (0.8 cc. removed with a minimum of disturbance)	28.5	5	7.5	8	5.5	54.5
	0.5 Cc.					
	1 (Original Dish)	2				Total Killed
B_1 (fluid mixed before removal of 0.5 cc.)	22	25				47
B_2 (0.5 cc. removed with a minimum of disturbance)	39	17				56
C (1 cc. of fluid left undivided)						56.5

B_1 and B_2 were similar to A_1 and A_2, except that the fluid was divided into halves instead of fifths. In test C the 1 cc. of fluid was left undivided. Two experiments of this kind were run, the results of the second completely confirming those of the first. Table 5 gives the averaged results of these two sets of tests. When the fluid was thoroughly mixed, the paramecin was fairly evenly distributed through the smaller equal fractions was withdrawn. In all cases, whether the fluid was mixed before division or not or whether the fluid was not divided at all, the total number killed approximates the number (50) of killers that had been left in the 1 cc. of fluid for the 5-hour period. These results justify the conclusion that the paramecin tends to settle on the bottom of the culture dish. This might mean that the paramecin particle itself is large or that the paramecin is adsorbed to

something else that carries it to the bottom. In relation to this tendency of the paramecin to collect on the bottom of the dish, it should be mentioned that Sonneborn, Jacobson, and Dippell (1946) have discovered that, after concentrates of the broken bodies of killer paramecia are centrifuged at seventeen hundred times gravity for 10 minutes, higher titers of paramecin are found in the centrifugate than in the supernatant fluid.

The fact that, under the conditions of these experiments, the paramecin collects, at least in large measure, on the bottom of the dish gives a reasonable explanation for the ease with which paramecin is removed with large numbers of killers from a small and shallow quantity of fluid in a test. It need hardly be pointed out that these two experiments still further confirm the general conclusions with regard to the relation of killers to killed and to the fairly regular and constant average rate of production of paramecin during the 5-hour interfission period of the killers.

5. *At what stage of the interfission period is paramecin liberated?*—The next question that seemed of interest to investigate was whether there was any particular part of the interfission period that was characterized by a greater production of paramecin than were other parts. Is the period immediately following or immediately preceding a fission one in which the paramecia are more active in the production of paramecin than in the intermediate ones, or may it be produced at any time during the interfission period? Is more paramecin formed than remains effective throughout the 5-hour period?

The answers to such questions as these were sought through a number of experiments in which the 5-hour interfission period was broken up into shorter periods, with tests made to determine the amount of paramecin in the fluid in which the killers had lived for such shorter periods. Recently divided killer paramecia were introduced into fluid and allowed to remain there for one-third, one-fifth, or one-ninth of the interfission period of 5 hours. They were then removed and introduced into a new volume of fluid for an equal period of time. The process was repeated until the whole interfission cycle of 5 hours had been completed. Each time the killers were withdrawn from a volume of fluid, 100–200 sensitive animals were added to the fluid, and observations were made on the three following days to determine the number killed in each. The 5-hour period was divided into only three parts in 27 experiments: in 24 of them the paramecin production of a single killer animal was observed; in 3 of them the paramecin production of eight killer animals was studied. In 16 other experiments the 5-hour period was divided into five 1-hour periods: in 9 of these experiments, single killers were used; in the other 7, ten killers were used in each. In a third group of experiments the interfission period was subdivided into nine periods of approximately 35 minutes each, with 3 experiments run with a single killer in each, 2 with four killers, and 1 with eight killers. Table 6 gives the results of these three series of experiments.

a) Can one killer kill more than one sensitive individual in 5 hours? It was mentioned in Section II that there were indications that it can but that these were somewhat confused by the fact that sometimes a lethally affected individual may undergo an abnormal division and give rise to two corpses. In the tests with single killers made in these three experiments, there is no possibility of misinterpretation, for, when there were multiple deaths from one killer, they occurred not in the same test but in separate tests of different subdivisions of the

5-hour period. Thirty-six tests were made with single killers. In 4 of these tests there was no killing at all; in 23 of them one sensitive animal was killed during the 5 hours; in 8 cases a sensitive was killed in two of the subdivisions of the interfission period; in 1 case there was the killing of a sensitive in three of the subdivisions.

is higher than 1; in two series it is exactly 1; in two series it is less than 1. It was rather surprising to find so little evidence of loss of paramecin in 5 hours' time at 27° in these experiments. Sonneborn, Dippell, and Jacobson (1947) discovered that 90 per cent of the paramecin that is released from the disintegrated bodies of killer animals is lost in 8 hours at 32°, in

TABLE 6

KILLING ACTION OF FLUIDS IN WHICH KILLERS LIVED FOR VARIOUS SUCCESSIVE SUBDIVISIONS OF THE 5-HOUR INTERFISSION PERIOD

In each case, fluid A is fluid in which the killers lived for the first period after fission; fluid B is fluid to which they were then transferred for an equal period, etc., until a full interfission period of 5 hours had been completed. After removal of the killers, sensitives were added to the fluid to ascertain the number that would be killed.

Length of Time Each Successive Fluid Was Occupied by Killers (Minutes)	No. of Experiments	No. of Killers per Experiment	No. of Sensitives Killed by Successive Volumes of Fluid									Total No. of Sensitives Killed	Mean No. of Sensitives Killed per Killer
			A	B	C	D	E	F	G	H	I		
100	24	1	7	9	11	....	....	....	....	....	....	27	1.1
	3	8	1	11	4	....	....	....	....	....	....	16	0.6
Total killed	.....	.....	8	20	15	....	....	....	....	....	....	43	0.9
60	9	1	1	1	3	2	5	....	....	....	....	12	1.3
	7	10	20	22	16	8	10	....	....	....	....	76	1.0
Total killed	.....	.....	21	23	19	10	15	....	....	....	....	88	1.1
35	3	1	1	1	0	1	0	0	0	0	0	3	1.0
	2	4	2	1	2	1	1	1	1	2	1	12	1.5
	1	8	1	0	1	0	0	1	1	0	1	5	0.6
Total killed	.....	.....	4	2	3	2	1	2	2	2	2	20	1.0

b) Is there any indication that more paramecin is formed than remains effective throughout the 5-hour period? The fact that the relationship between the number of killers and the mean number killed during the entire 5-hour period was still close to a 1:1 relationship, as it had been in experiments with the 5-hour period undivided, would seem to indicate that under these conditions little, if any, of the paramecin disintegrates or becomes ineffective in any other way during the time between divisions. In three series the mean number killed per killer

27 hours at 21°. Sonneborn, Jacobson, and Dippell (1946), to be sure, found a rise in titer of such concentrates for a few hours at room temperature before there was a decline. It is not known whether the basis for the seeming discrepancy between the results of the present experiments and those of Sonneborn, Dippell, and Jacobson lies in some such exceptional condition at 27°; in important differences in technique (since in the experiments with paramecin concentrates the fragments of broken bodies of paramecia are present, while in these experiments

they are not); or in some other as yet unidentified factor. In recent extensive studies of paramecin inactivation, van Wagtendonk and Zill (1947) have found that it is influenced by hydrogen-ion concentration as well as by temperature.

c) May paramecin be produced at any time during the interfission period, or is there some subdivision of the period in which it is produced more abundantly than in others? The table makes it clear that, on the whole, there is equal probability that the paramecin that is formed as the result of the activity of each killer animal between divisions may be formed in any one of the shorter periods, among those tested, into which the 5-hour period is subdivided.

6. *Discussion of results in Section A.*— The results up to this point agree in showing that, on the average, each killer liberates into the culture medium in 5 hours enough paramecin to kill one sensitive animal. The data further show that this quantity of paramecin behaves as a discrete unit-particle that is either entirely present in any portion of a volume of fluid in which killers have lived or entirely absent from it. The approximate 1:1 relation between the number of killers and the mean number killed, while not essential to the hypothesis that one particle can kill one animal, did show that the rate of production of paramecin is fairly constant, that the response of the sensitive stock 31 to the paramecin produced in an interfission period is fairly constant (under the conditions of these experiments), and that therefore the number of killers present could be used as a direct, if approximate, measure of the amount of paramecin produced. This, in turn, led to the suggestion that, on the average, the amount of paramecin produced by a killer in 5 hours is one unit-particle.

This suggestion is of such great interest and importance that it seemed desirable to test it in a number of ways. Sonneborn, Dippell, and Jacobson (1947) have tested it by the use of certain methods, the present author by the use of others. The following section gives the results of a series of experiments in which the number of sensitives used to detect the paramecin was varied with each constant quantity of paramecin. This method gives a means of testing the one-particle hypothesis, because the theoretical expectations of the numbers that would be killed as the number of sensitives increases are different on the two following assumptions: (1) that one particle kills one sensitive and (2) that more than one particle is necessary in order to kill one sensitive. If one particle kills one sensitive, it would be expected that, when a constant quantity of paramecin is used, the number killed would remain approximately the same as the number of sensitives increases. If more than one particle is necessary in order to kill one sensitive, it would be expected that, as the ratio of the number of sensitives to the number of paramecin particles increases, the chance that any sensitive animal in the test can come into contact with more than one particle would decrease and that therefore the number of sensitives killed would, in general, decrease.

B. THE NUMBER OF SENSITIVES KILLED WHEN AT EACH CONSTANT LEVEL OF PARAMECIN THE NUMBER OF EXPOSED SENSITIVES IS VARIED

1. *Results of experiments.*—In all the experiments described in Part A, the number of sensitive animals was kept approximately the same in each test, usually about 100–200 per 0.1–0.2 cc. As stated above, in the present series of experiments the number of sensitive animals was varied with each constant level of paramecin dosage. As before, 1, 2, 4, 8, 16, and 32 killers were allowed to live in

0.1 cc. of fluid, and, after their removal, 0.1 cc. of a culture of sensitive animals was added. The method employed in varying the population density of the sensitive culture was to concentrate the sensitive animals by centrifuging for from 4 to 5 minutes at about 2,000 r.p.m. The approximate number of animals per 0.1 cc. of fluid was determined by sample counts, and this concentrated culture of sensitives was then diluted to various concentrations with the supernatant fluid. Concentrations below 100 were obtained accurately by introducing with a fine capillary pipette the desired numbers of sensitives into 0.1 cc. of the supernatant fluid. In these two ways concentrations varying from 4 to about 2,400 sensitive animals per 0.1 cc. were obtained.

When 4, 8, 16, 32, or 64 sensitives were used, the average numbers killed in four experiments were always less than the number of killers. There was considerable variation, but, in general, the mean number killed per killer decreased with a decrease in the number of killers from 0.6 with 32 killers to 0.02 with 1 killer and decreased with a decrease in the number of sensitives from 0.5 with 64 sensitives to 0.05 with 4 sensitives. This might have been predicted from the fact that sooner or later paramecin does in some way become inactivated (Sonneborn, 1939; Sonneborn, Jacobson, and Dippell, 1946; Sonneborn, Dippell, and Jacobson, 1947). With low concentrations of sensitives, it would take an increased length of time for paramecin particle and animal to connect by chance. Hence much paramecin could become inactivated before it had come in contact with an animal. Moreover, when only a few sensitives are present, the chances for a single sensitive to take up more than the minimum amount of paramecin are greater than they are when more sensitives are used, and thus some paramecin might be "wasted" in this way.

It is of interest to contrast these results, obtained when low concentrations of sensitives are used, with those of various authors who have studied the effects of group protection from toxic agents, already mentioned in another connection in Section A, 3 (see Allee, 1931, or Fowler, 1931 for bibliographies on the subject). Allee (from 1920 on) and numerous others since 1920 have found that, in general, when the volume and the concentration of the toxic substance are constant, the smaller the mass of animals, the poorer is their protection. No such group protection is evident in these experiments in which, in a constant volume of fluid, the number of sensitives is varied for each level of paramecin concentration. Exceptional cases in which single individuals lived longer than groups were found by Fowler (1931) in the more dilute concentrations of the toxic substances he used and were explained by him on the basis of Child's theory of acclimation of animals in weaker solutions (for a discussion and bibliography of this subject see Child, 1941), an acclimation which, Fowler found, goes on better in the absence of carbon dioxide and therefore better when fewer animals are present. No direct tests for such acclimation have been made in these experiments on the effect of paramecin on small numbers of sensitives, but the evidence at hand does not indicate its occurrence. It is possible that a dissimilarity, such as this appears to be, between the reaction of animals exposed to paramecin and that of animals exposed to toxic solutions of various sorts might furnish a negative, if not a positive, clue as to the nature of paramecin.

The averaged results of four experiments in which the number of sensitive animals was varied from 120 to 2,400 for

each level of paramecin concentration used are shown in the upper half of Table 7. Two important points are made clear by the results shown here: (1) The mean number killed per killer is again approximately 1. It is interesting that the discrepancy from the 1:1 relation between the number of killers and the number killed may be accounted for almost entirely by the results with 32 killers. The reason for this has already been discussed in Sections A, 3 and 4. (2) Varying the number of sensitives between 120 and 2,400 has no effect on the number of sensitives killed.

2. *The one-particle versus the more-than-one-particle hypothesis.*—These results provide the strongest type of evidence for the validity of the hypothesis that a sensitive animal can be killed by one unit-particle of paramecin. If one particle of paramecin is sufficient to kill a sensitive cell of stock 31, then obviously there will always be one animal killed

TABLE 7

KILLING ACTION OF 1–32 KILLERS WHEN THE NUMBER OF SENSITIVE ANIMALS IS VARIED FROM 120 TO 2,400 PER 0.2 CC.

For comparison with the observed values, the theoretical expectations on the hypothesis that two or more particles are required to kill a sensitive animal are included from calculations based on the Poisson series. On the one-particle hypothesis, the number killed would closely approximate the number of killers.

No. of Killers	No. of Sensitives per Test				
	120	240	600	1,200	2,400
	Observed Results of 4 Experiments: Mean No. of Sensitives Killed				
1	1	1	1	1	1
2	2	2	3	2	2
4	4	4	4.2	4.5	4
8	9.5	6.6	8.6	10.3	10
16	16	14.1	16.2	12.8	15
32	25	21.3	22	21.5	24
Total no. killed	57.5	49.0	54.0	52.1	56.0
No. of sensitives killed per killer: mean of means	0.9	0.9	1.0	0.9	0.9
	Theoretical Expectations on the Hypothesis that Two or More Particles Are Required To Kill a Sensitive Animal Mean No. of Sensitives Killed				
1	14	9	3	2	1
2	40	29	14	7	4
4	82	81	50	40	14
8	115	166	148	100	56
16	120	230	342	298	200
32	120	239	537	685	596
Total no. killed	491	754	1,094	1,132	871
No. of sensitives killed per killer: mean of of means	12.6	14.4	13.1	11.3	7.4

when one particle is present in a culture of this stock, regardless of the number of sensitive animals present, if only the number is large enough to insure contact between the paramecin particle and some animal before the paramecin becomes inactivated.

But let us consider what the expected results would be on the basis of alternative interpretations. Suppose, for example, that at least two particles of paramecin were necessary to kill one sensitive animal. To insure a reasonable chance that two particles would come into contact with a particular animal, it would be necessary to have a considerably larger number than two. When, for example, 2,400 sensitive animals are present in a volume of fluid containing thirty-two particles of paramecin, the chances of two or more particles meeting one animal are only about one in ten thousand. As the number of sensitive animals decreases, the ratio of paramecin particles to sensitive animals increases, and, correspondingly, the chances of two or more particles being picked up by one animal become better and better down to the point where, as has been pointed out, some particles will disintegrate or become inactivated in some other way before coming into contact with any animal at all. Therefore, we should expect that down to that point the percentage killed would, in general, rise as the number of sensitive animals in the test decreases.

The theoretical expectations for the numbers of animals that would be killed on the hypothesis that more than one particle is required to kill one animal can be calculated by the use of the Poisson series. These expected values when 120, 240, 600, 1,200, and 2,400 sensitive animals are used are given in the lower half of Table 7. The first two terms of the Poisson series, e^{-m} and m/e^{m} (where e is the base of the natural logarithms, 2.71828, and m is the mean—in this case the mean number of particles of paramecin per sensitive animal), give the fractions of animals that would by chance come into contact with no particles and with one particle, respectively. The remainder of the animals, or

$$1-\left(e^{-m}+\frac{m}{e^{m}}\right),$$

would then come into contact with two or more particles of paramecin. The basis for the calculations resulting in the values given in Table 7 was the observed fact that, in the combination of 1 killer and 2,400 sensitives, 1 sensitive (i.e., about 0.04 per cent of the total number) was killed (see upper half of the table). According to the Poisson series, this is approximately the percentage that would be expected to get two or more particles if 0.03 is the mean number of particles per sensitive animal, for, with this mean, 99.96 per cent of the animals would get either no particles or one particle each. This mean of 0.03 then was the mean assumed for the combination of 2,400 sensitives and 1 killer. The total number of particles in the test would be 2400×0.03, or 72 particles. From this information the mean number of particles per sensitive animal is readily computed for each of the other cases in the table. Cases involving two killers may be assumed to have twice as many total particles of paramecin as those with one killer, viz., 144 particles; those with four killers four times as many, or 288; etc. It should be pointed out that the theoretical values obtained depend upon which observed result forms the starting-point for the computations. Any one of the other observed results could have been arbitrarily selected for this purpose, and the theoretical values would then have been different. But, no matter which one served as

the base for the calculations, the relations of the values to one another would always be different from those shown in the upper half of Table 7, where the number killed varies with the number of killers but shows extraordinary constancy when the number of sensitives is varied. On the hypothesis that more than one particle is necessary to kill, for any constant number of killers the calculated number of sensitives killed would always vary greatly with a variation in the number of sensitives, as it does for the case shown in the lower half of Table 7.

Before leaving this matter of the theoretical calculations made by the use of the Poisson series, it should be explicitly stated that two fundamental assumptions are made which may not be strictly true but which seem at least to be near the truth. The first of these is that, with the numbers of sensitives employed, all particles of paramecin make contact with sensitive animals. The second of these is that the particles of paramecin are randomly distributed with respect to the sensitive animals or, perhaps more accurately, that the rapidly moving sensitive animals are randomly distributed with respect to the paramecin particles, many of which may be more or less stationary on the bottom of the culture dish unless disturbed. This settling of the paramecin on the bottom, the evidence for which is discussed in Section A, 4, would have no more influence on the random connection of animal and particle than the stationary position of the card players would have in the random distribution of cards!

If it was assumed that it took more than two particles to kill a sensitive cell, then the discrepancies between the theoretical calculations and the observed results in the upper part of Table 7 would be even greater than those shown. But on the one-particle hypothesis the expected values and relations between the values as the number of killers is increased or as the number of sensitives is increased are in close agreement with the observed values and relations as shown in Table 7. The expectations on the one-particle hypothesis are (1) that a fairly constant number would be killed at each level of paramecin concentration, regardless of the concentration of sensitives; (2) that, if it is assumed that each killer is, on the average, responsible for the liberation of one particle of paramecin in each 5-hour period, the number killed would be approximately the same as the number of killers, regardless of the concentration of sensitives; and (3) that there would be an approximate doubling of the number killed as the number of killers is doubled, regardless of the concentration of sensitives. With all three of these expectations realized, evidence for the validity of the single-particle hypothesis becomes very strong.

IV. SUMMARY AND CONCLUSIONS

A. SUMMARY OF RESULTS

1. In *P. aurelia*, when 1, 2, 4, 8, 16, and 32 killers of stock 51 of variety 4 are introduced into fluid with a constant number of animals of three different sensitive stocks—29 and 32 of variety 4 and 31 of variety 8—and allowed to remain, at the end of 24 hours there is an approximate doubling of the number of sensitives killed as the number of killers is doubled in all three stocks. But, for any "dosage" of paramecin, in stock 29 only about half as many animals are killed as in stock 31, while stock 32 gives intermediate results. It is planned to investigate the causes of the differences later. The finding of a fairly constant relationship between the number of killers and the number killed in these preliminary experiments led to the study of this relation-

ship under more carefully controlled conditions with only stock 31 as the sensitive material.

2. With stock 51 of variety 4 used as killer animals and stock 31 as sensitive material, the following facts have been found:

a) When the number of killer animals in a volume of fluid for a unit time-period is doubled, the number of sensitives that are killed by the fluid is also doubled.

b) One killer produces in 5 hours enough paramecin to kill, on the average, one sensitive animal.

c) This paramecin is produced as a single unit, for if one killer is kept in different samples of fluid in successive fractions of the 5-hour interval, any one sample either has the full amount of paramecin or none at all. As a rule, one, and only one, of the samples in such a series contains detectable paramecin, and this one has the minimal detectable amount—enough to kill one sensitive animal.

d) Evidence that paramecin tends to collect on the bottom of the culture dish was furnished by a comparison of the killing action of portions of paramecin fluid removed after thorough mixture and portions carefully removed without such mixture. When the fluid is thoroughly mixed before removal, the killing action of equal subdivisions (fifths in two experiments, halves in two others) of 1 cc. of fluid is about the same. When the fluid is removed with as little disturbance as possible and without mixture, 50 per cent of the total number of sensitives killed may be killed in the original dish from which four-fifths of the fluid has been removed, or 70 per cent in the original dish from which half has been removed.

e) When the number of exposed sensitive animals is varied at each constant level of paramecin concentration, a fairly constant number of sensitives, closely approximating the number of killers, is killed at each level of paramecin concentration, regardless of the concentration of sensitives (between 120 and 2,400 per 0.2 cc.). Also regardless of the number of sensitives (above 120), there is an approximate doubling of the number killed as the number of killers is doubled.

B. CONCLUSIONS

These results lead to the following three main conclusions, the first and last of which have also been reached on the basis of different types of experiments by Sonneborn, Dippell, and Jacobson (1947):

1. Paramecin occurs in the form of unit-particles.

2. Under the conditions of these experiments, killers are, on the average, responsible for the production in the culture medium of one unit-particle of paramecin per killer animal per 5 hours.

3. A sensitive paramecium can be killed by a single unit-particle of paramecin.

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